Medical Library

JOURNAL OF ANATOMY

ORIGINALLY THE JOURNAL OF ANATOMY AND PHYSIOLOGY

CONDUCTED ON BEHALF OF THE ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND BY

J. D. BOYD
J. KIRK (TREASURER)
C. M. WEST
F. WOOD JONES
W. E. LE GROS CLARK (ACTING EDITOR)

VOLUME 76
PART 2
JANUARY 1942

Entered at New York Post Office as Second Class Matter

(All rights reserved)

CAMBRIDGE UNIVERSITY PRESS
LONDON, BENTLEY HOUSE

CHICAGO: The University of Chicago Press
(Agents for the United States)
BOMBAY, CALCUTTA, MADRAS: Macmillan
TOKYO: Maruzen Company Ltd.

All rights reserved

Price Twelve Shillings and Sixpence net

PRINTED IN GREAT BRITAIN

DEVELOPMENTAL ABNORMALITIES OF THE EYE

By IDA C. MANN. 50s. net

284 illustrations in colour and black and white

With a Foreword by SIR JOHN H. PARSONS

Published for the *British Journal of Ophthalmology*

This book is the logical successor to Miss Mann's earlier book "The Development of the Human Eye." It applies a knowledge of embryology to the elucidation of clinical problems, and is mainly, though not entirely, based on Miss Mann's practical experience.

CAMBRIDGE UNIVERSITY PRESS

ORGANISERS & GENES

By C. H. WADDINGTON

2 plates. 12s. 6d. net

A new volume in the series of *Cambridge Biological Studies*

Development is a fundamental property of all animal structures and functions. The discovery of the organiser has in recent years opened the way to a much deeper causal analysis of development; and the science of genetics has shown that the factors which ultimately control developmental processes are the genes. This book attempts, firstly, to analyse the significance of the organiser phenomenon for the general theory of development, and secondly to exhibit the similarities between it and gene action. The first part of the book contains a survey of organisers in different groups, and a discussion of the concepts of evocation, individuation and competence, as three fundamental problems which are raised by the consideration of the organiser reaction. A discussion of the genetic data bearing on these problems leads to a consideration of the formation of animal shapes and patterns. The last chapter is devoted to an evaluation, in relation to the previous discussion, of some general biological concepts such as fields and organisation.

CAMBRIDGE UNIVERSITY PRESS

THE DEGENERATION AND RE-INNERVATION OF GRAFTED NERVES

By F. K. SANDERS[1] and J. Z. YOUNG[2]

Department of Zoology and Comparative Anatomy, University Museum, Oxford

INTRODUCTION

Since the classical experiment of Phillipeaux & Vulpian (1870), many authors (e.g. Huber, 1895, 1920; Cajal, 1928; Ballance & Duel, 1932 *a*, *b*) have shown that grafted pieces of nerve may be used to conduct new fibres across the gaps caused by lesions of peripheral nerves. Various types of nerve graft have been used. Autografts (pieces of nerve from the same individual) are reported by many authors as giving successful recovery of sensation and motor function both in animals and man (see Kilvington, 1908; Eden, 1919; Huber, 1920; Cajal, 1928; Duel, 1933; Bentley & Hill, 1936; Bunnell & Boyes, 1939). The value of homografts (pieces of nerve from another individual of the same species) has been less thoroughly tested, but Kilvington (1908), Eden (1919), Huber (1920), Bentley & Hill (1940) all report successful results following homografts in animals. Heterografts (the transplantation of pieces of nerve from one species to a different one) have never been fully investigated. Huber (1895) reported success in grafting the sciatic nerves of cats into dogs, but later (Huber, 1919, 1920) suggested that heterografts are less successful than homografts or autografts. In fact, he says that in heterografts 'many of the downgrowing neuraxes pass outside the nerve transplant and thus reach the distal nerve segment'. Cajal (1928) condemns the use of heterografts, as does Eden (1919) on the basis of his own experiments and a review of previous work.

Various methods of treatment prior to the insertion of the graft have been proposed. In the case of autografts, pre-degeneration of the grafted segment has been proposed by Cajal (1928) on theoretical, and by Duel (1933) on experimental, grounds. Storage of homografts and heterografts on ice (Bethe, 1916), in saline (Tello, 1915), in a 3 % aqueous solution of borax + a few drops of formalin (Bielschowsky & Unger, 1917) in vaseline or liquid petrolatum (Huber, 1920), has been tried with some success, but the possibilities of nerve storage have never been fully explored. Nageotte (1917 *a*, *c*) has advocated the use of dead grafts preserved in 50 % alcohol, and claims great success in their use, which is supported by the experiments of Huber (1920), but not by the experience of clinicians (see Laduron & Christophe, 1938). Gosset & Bertrand (1938) have made use of heterografts of dog and rabbit spinal cord fixed in formol and stored in alcohol. Cajal (1928), however, states that grafts treated by boiling

[1] Harmsworth Senior Scholar of Merton College, Oxford.
[2] Fellow of Magdalen College, Oxford.

water, chloroform, formalin, or chloral hydrate, are not innervated, and the use of dead grafts still remains a controversial point.

The results of nerve grafting in clinical cases are extremely difficult to estimate, on account of the differing methods used to make the graft, and the varying standards adopted for estimating recovery. However, the extreme pessimism of British surgeons on the whole subject of nerve grafting in man (see *M.R.C.* report, 1920; Platt, 1919; Stopford, 1920; Sargent, 1920) is hardly justified. Foerster (1929), out of 21 autografts, had 5 complete recoveries, 12 ameliorations, and only 2 failures; 2 cases were not followed up. Bunnell & Boyes (1939) report 32 cases of autografting in the small nerves of the hand in which there was a high proportion of recoveries, while the thin Ballance-Duel grafts used in cases of facial paralysis show remarkable success.

The great difficulty in assessing the results of all this experimental and clinical work on the subject is that no *measure* of the efficacy of the various types of graft has been devised. Each author has claimed that some particular method of grafting gave success, but there has been very little comparison between methods on the basis of standard criteria. Moreover, there is very little information as to the nature of the histological changes which proceed in the various grafts—especially during the early stages.

The experiments described in this paper represent an attempt to compare the efficiency of the following types of nerve graft:

(*a*) Fresh autografts.
(*b*) Pre-degenerated autografts.
(*c*) Fresh homografts.
(*d*) Homografts stored in Ringer's solution at 2° C.
(*e*) Fresh heterografts.
(*f*) Dead homografts preserved in 50 % alcohol.
(*g*) Dead heterografts of alcohol-preserved spinal cord.

The comparison has been made by estimating the distance which new fibres had grown through the graft after short periods (mostly 15 and 25 days) and by careful examination of the histological conditions at these times. Such criteria are of course in many ways inferior to the test of functional recovery (which is, however, now also being applied by one of us—F. K. S.). But the use of short periods has the great advantage that it allows the investigation of numerous animals in a reasonable time, and thus avoids what we feel to be the main defect of earlier work—namely that, being neither statistical nor quantitative, it provides no basis for induction. The animals used were rabbits and the problems of comparing results so obtained with conditions in man are discussed on p. 160.

METHODS

The standard method used for assessing the value of the various types of graft is that described by Young *et al.* (1940). Grafts were 2 cm. in length, and were used to fill gaps in the resected peroneal nerve of the rabbit.

In an initial aseptic operation under anaesthesia (nembutal and ether) grafts of two different sorts were inserted in the peroneal nerve on opposite sides of the same rabbit, and fixed in position with concentrated fibrinogen solutions in cockerel plasma as described by Young & Medawar (1940). These solutions proved very convenient for the fixing of grafts, healing of the graft into the host nerve normally taking place before the clot of plasma was resorbed. By making a suitable gap of slightly less than 2 cm. in the host nerve, retraction and consequent scarring at the junctions were avoided. A standard junction was thus obtained in which there was no suture material to obstruct the downgrowth of new fibres from the central stump.

At either 15 or 25 days after operation the grafts in each animal were exposed under light anaesthesia, and the distance reached by outgrowing sensory fibres estimated. This was done by freeing the nerve from its bed, and then pinching with fine forceps every 2 mm. or so from a distal point upwards until a reflex response was obtained. Histological study of regions where the first reflex response to such pinching was obtained has shown that this simple method gives a very reliable estimate of the distance reached by (presumably) pain fibres.

After the distances of outgrowth had been measured, the grafted pieces were removed and fixed for sectioning and staining by various histological methods. The routine methods were, for axons, the Bodian silver proteinate, alone or with Masson or Mallory trichrome stains, and for myelin, Weigert-Pal and various osmium tetroxide methods.

AUTOGRAFTS

Fresh autografts. Sixty-three fresh autografts were made in rabbits, each graft consisting of a 2 cm. length of the tibial nerve inserted into a gap made in the peroneal. These two nerves lie side by side in the thigh, and only one skin incision was thus necessary both to take the graft and to insert it. The tibial nerve was used as a free graft, being entirely removed before insertion, and, since it is the thicker of the two, it offers full opportunity for the outgrowth of all the peroneal fibres. In some experiments the graft was inserted so as to maintain its original polarity; in others the polarity was reversed. There was no outstanding difference in the distances of outgrowth obtained between those grafts in which the polarity was reversed and those in which it was unchanged, nor between grafts into the peroneal of the same or the opposite side of the animal.

In animals opened at both 15 and 25 days autografts had always become well united with the host peroneal nerve, and had an appearance not unlike normal nerve. When pinched with forceps, such grafts did not feel hard or 'doughy', as in the case of some other types (see below). The junctions were only occasionally bulbous, and the grafted pieces retained the dimensions which they had at the initial operation. There were occasionally adhesions between the graft and its bed, although when these occurred they were

confined principally to the junctions. Any adhesions, however, were always markedly less than with fresh homografts (see below). At 15 days autografts had usually already established a vascular supply, and this came invariably from along the nerve, usually from vessels in the popliteal space. There was never an encapsulation of the graft as in the case of other types of graft (see below).

In three animals at 25 days the distal junction was found to have become detached subsequent to operation, and in each of these cases the distal end of the graft was found joined to the peripheral stump of the peroneal nerve by strands of proliferated tissue, originating partly from the graft and partly from the peripheral stump. In some cases these 'spontaneous' junctions had been penetrated by downgrowing fibres which had reached the peripheral stump by this route. Junction between a detached autograft and the peripheral stump can thus take place in a manner strictly comparable to that described by Kirk & Lewis (1917) for resected nerves which were not sutured.

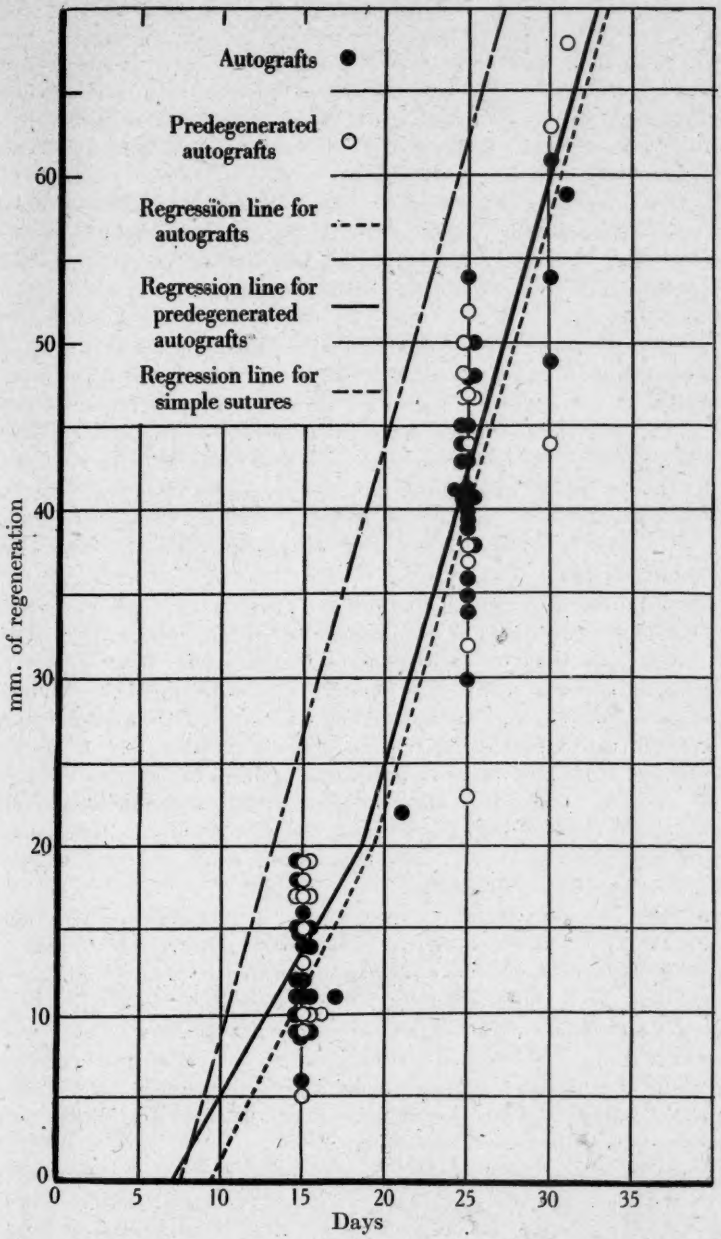
Autografts examined at 68 and 102 days still preserved the above distinctive features. There was no sign of the conversion of the graft into a connective tissue strand which has been stated to occur in man (see Stopford, 1920; Platt, 1921).[1]

Text-fig. 1 shows the distances reached by outgrowing fibres after fresh autografting. At 15 days all fibres were still in the graft, but at 25 days fibres had passed the distal junction and grown on the average 41·7 mm. from the upper junction, that is, 21·7 mm. into the peripheral stump. New fibres grow through autografts only slightly more slowly than through a normal peripheral stump. The data of Young & Medawar (1940) give the average rate of growth of pain fibres down a normal peripheral stump as 3·9 mm./day after plasma junction, with a latent period of approximately 8 days before the fibres cross the scar.[2] In the case of autografts the present data for distances reached at 15 and 25 days give an estimate for the latent period of about 9·2 days and a rate of growth of 2·0 mm./day. These rates are calculated on the assumption that growth through the peripheral stump occurs at 3·5 mm./day, i.e. that growth across the upper junction and in the graft occupies $25 - \frac{41{\cdot}7 - 20}{3{\cdot}5} = 18{\cdot}8$ days. The further assumption that there is no delay at the lower junction is discussed on p. 148.

Histology of fresh autografts. Degeneration and re-innervation proceed in fresh autografts almost exactly as in a normal peripheral stump. Proliferation of the Schwann nuclei and break-up of the myelin into ovoids proceed actively (see Pl. 1, figs. 6–8). The graft is invaded by macrophages which remove myelin remains as they do in a normal stump, although the process is rather

[1] Recently a piece of autografted nerve was removed by Prof. H. J. Seddon 5 months after insertion into a human median nerve. Histological examination showed that normal nerve structure was maintained.

[2] These figures have now been revised by additional data to 3·5 mm./day + 7·3 days.



Text-fig. 1. Distances at which reflex responses were obtained on pinching the peroneal nerve after insertion into it of pieces of tibial nerve 20 mm. long from the same animal. The lines are the regression lines, arithmetically calculated from the results at 15 and 25 days only. For comparison the line showing the rate of growth through a normal peripheral stump is added from the data of Young & Medawar (1940). The times of pre-degeneration varied from 6 to 28 days.

slower. After 25 days a section from an autograft can usually be distinguished from a normal stump by the fact that spaces in which the myelin remains lie are abnormally large (Pl. 1, fig. 7). It is not possible to say whether this condition is likely to prejudice recovery, but it may cause some of the fibres to run irregular courses.

No sign of necrosis at the centre of these grafts was seen. The tissue had been well vascularized by 25 days, but the smaller vessels were often more dilated than in a normal stump (Pl. 1, fig. 6), and occasionally there was a small lymphocytic reaction around some of the vessels (Pl. 1, fig. 7), but this was usually absent.

Junction with the central and peripheral stumps is made in a manner indistinguishable from a normal union of stumps. In particular it must be noted that the lower junction is made by the Schwann cells (presumably both those of the graft and of the peripheral stump) *before* the arrival of axons from the central stump, the union being already well made after 15 days (Pl. 2, fig. 9). This process of union is, in fact, *independent of the presence of axons*, and there should be no grounds for the fear expressed by some (e.g. Davis & Cleveland, 1934) of the delaying, and perhaps obstruction, of fibre growth on account of fibrosis at the distal junction.

The distances of outgrowth obtained at 15 and 25 days also indicate that there can be no great delay at the distal junction. The rates of growth given on p. 146 are calculated on the assumption that this delay is zero, and yet the difference in estimated rate of growth of fibres through autografts and normal peripheral stumps is only between 2·0 and 3·5 mm./day. If we assumed that the *whole* of this apparent difference is actually due to delay at the lower junction, i.e. that the actual rate of growth of fibres in both graft and peripheral stump is 3·5 mm./day, then the delay at the lower junction would be only 1·7 days. These figures support the histological findings in the conclusion that the peripheral junction is made before fibres reach it, so that when they arrive they can immediately cross.

In order to confirm this for longer grafts, in two experiments grafts of tibial nerve 5 cm. long were inserted in the peroneal nerve. The animals were reopened at the time when, by calculation from the above rate of fibre growth through autografts, fibres should have crossed the lower junction. In both cases this had indeed occurred, and fibres had travelled some way into the peripheral stump. In addition the histology of the distal junction of these long grafts, in which any 'fibrosis' should have been specially marked, showed no greater degree of fibrosis or whorling of fibres than occurs with the standard 2 cm. grafts described above (see Pl. 2, fig. 10).

There have been cases, however, in which both lower and upper junctions were not made quite closely, there being a gap of 1 or 2 mm., with considerable resultant criss-crossing. This is probably due to the slight shrinkage in length which is apt to occur in the graft, and it may be that the obstructions seen by Davis & Cleveland were produced in this way. However, it is not clear from

their paper exactly what these authors mean by the 'fibrosis' which they claim to occur. The only method of staining which they mention (Ranson's) would be suitable only for the study of axons. Their figures do not enable a comparison to be made between scars after primary and secondary suture, since those of the latter are at a much higher magnification. Thus the postulation of obstruction of fibre growth at the distal scar, which has led some to suggest a practice of severing and resuturing the lower junctions of long grafts, is still not proved by experimental evidence. This procedure might, however, become necessary if the lower (or upper) junction became detached owing to shrinkage of the graft or other causes. But figs. 9 and 10 on Pl. 2 show that under the best conditions perfectly smooth primary lower junctions can be made.

Re-innervation proceeds uniformly over all parts of these grafts. Great numbers of fibres can be seen after 15 and 25 days growing down the degenerating Schwann bands in a normal manner. There is no sign of a restriction of the innervation to the edges of the graft. Moreover, after 25 days the new fibres are becoming medullated, again in all parts of the grafts (Pl. 1, fig. 8, and Pl. 2, fig. 11). This must be an indication of the presence of live, healthy, Schwann cells. Indeed, all the evidence indicates clearly that a piece of nerve of the thickness of the rabbit's tibial is able to act as a graft in the fullest sense of the word, the grafted tissues surviving, becoming vascularized, and continuing to function in a manner approaching the normal.

Pre-degenerated autografts. A degenerated stump was prepared by cutting the tibial nerve at a preliminary operation, and leaving it for a time to degenerate. The central stump was resected for some millimetres at this operation, to avoid re-innervation of the peripheral stump during the time of degeneration. At the end of the degeneration time the animal was re-operated, and 2 cm. of the peripheral stump of the previously cut tibial was inserted into a gap in the peroneal of either the same or the opposite side of the animal. Thirty pre-degenerated autografts were made, and three sets of times of pre-degeneration were used: (1) 6–9 days, (2) 14–16 days, (3) 25–28 days.

In animals examined at both 15 and 25 days there was very little difference in superficial appearance between pre-degenerated autografts of all times of pre-degeneration and fresh autografts. Grafts were white or pink in colour, completely united with the peroneal, and well vascularized by vessels running along the nerve. When pinched, however, these grafts felt harder than fresh autografts. Bulbs did not regularly develop at either junction, but adhesions between the graft and its bed seemed to occur more frequently than in fresh autografts, and not to be confined to the junctions.

Text-fig. 1 shows the distances reached after 15 and 25 days by sensory fibres growing into pre-degenerated autografts. In some cases fibres have penetrated farther than in any fresh autograft, but the difference is not constant. In fact there is no difference in rate of fibre growth between fresh autografts and pre-degenerated grafts of any of the three times of pre-

degeneration, the calculated average rate in these grafts being 1·7 mm./day, with a latent period at the upper junction of 6·9 days, making the assumption of a rate of 3·5 mm./day in the peripheral stump and no delay at the lower junction. These figures are not significantly different from those for autografts, though the shorter latent period shown for pre-degenerated grafts is suggestive.

Duel (1933) in *Macaca* claimed a rate of regeneration through pre-degenerated grafts quicker by half than through fresh autografts. However, the numbers given are not adequate to support this contention and Bentley & Hill (1936) were unable to confirm it in cats. Since Duel's grafts were made in the facial nerve in the temporal bone, and were simply laid in position without suturing, they (Bentley & Hill) suggest that Duel's good results were due to the fact that the pre-degenerated grafts, being more rigid than fresh grafts, stayed in position better than the latter, and maintained better approximation at the junctions. Collier (1940) is also of this opinion. It is generally agreed that pre-degenerated nerve is easier to handle than fresh nerve, and for this reason may be very desirable in some situations.

Histology of pre-degenerated autografts. Histologically, pre-degenerated autografts showed no important differences from fresh autografts. The tissues of the graft survive, and the process of degeneration, begun during the period between the two operations, continues in a normal manner. After 25 days such grafts naturally show somewhat less myelin remains than do fresh autografts, and it is possible that this constitutes an advantage of pre-degeneration. With the times of pre-degeneration of the order used by surgeons and in the present experiments, this difference is not great, but it is just possible that much longer pre-degeneration times, say 2 months, might be an advantage for the grafting of thick nerves, which might survive and be re-vascularized more readily if the myelin has been removed, and when there is no active degeneration to make demands on the metabolism of the tissues. Only experiment can show whether this is so, or whether, conversely, as is possible, the active degeneration actually assists grafting by attracting new blood vessels.

The present results confirm those of Bentley & Hill (1936). Pre-degenerated autografts do not give a quicker regeneration than fresh autografts, and thus it is probably unnecessary for surgeons to go to the trouble of pre-degenerating grafts before use, except, perhaps, in the case of the facial nerve.

HOMOGRAFTS

Fresh homografts. Fresh homografts consisted of pieces of tibial nerve 2 cm. in length, taken from one rabbit, and used to bridge an appropriate gap in the peroneal nerve of a second animal. The two rabbits were opened at the same operation, and pieces of tibial nerve exchanged between them. Animals were examined at 15 and 25 days, and a total of thirty-five fresh homografts was made.

At 15 days fresh homografts showed considerable variability in appearance, although they were always completely united with the host peroneal nerve. Macroscopically, these grafts were either yellowish or reddish in appearance, and, in contrast to autografts, the graft and junctions were enclosed by a thick sheath with its own collateral blood supply. In contrast to this, vascularization of the graft itself was always along the nerve, and, although the extent of vascularization was very variable, it was always conspicuously less than in autografts. The formation of bulbs at the junctions was rare, but the grafts were often considerably swollen, the middle of the graft being blown out like a balloon. The occurrence of adhesions between the graft and its bed was also much more common than with fresh autografts.

At 25 days these general features of the appearance of fresh homografts were much accentuated: grafts were reddish or yellowish, enclosed in a thick sheath, and vascularized to a variable extent. Swelling of the graft, and adhesions, were commoner at 25 than at 15 days.

The variability of homografts shown in their appearance at biopsy appears again in the distances of outgrowth of fibres obtained at 15 and 25 days. These are shown in Text-figs. 2 and 3. At 15 days the distances at which reflex response to pinching was obtained vary from just across the proximal scar to 8 mm. past the distal scar in the peripheral stump. At 25 days they vary from only 6 mm. below the upper junction to 28 mm. below the lower one. Most of the animals at 25 days, however, gave distances between 14 and 22 mm.

When statistically treated, these results reveal a significant variability due to factors not under experimental control; a plot of error distributions reveals that the figures do not form a normally distributed population; indeed, there is a strong suggestion of two modes (Text-fig. 3). The mean distances of outgrowth have therefore little significance; they are, however, 15 mm. for 15 days, and 25·2 mm. for 25 days. It is not reasonable to obtain a value for the rate of growth through fresh homografts from these data. In favourable cases homografts give distances of outgrowth as large as those in fresh autografts (see Text-fig. 2). The main body of data, however, falls short of this. The great variability of the distances reached by fibres no doubt corresponds with the varying extent of the undesirable cell reactions described below.

Histology of fresh homografts. Degeneration and re-innervation can go on when a piece of nerve is grafted from one rabbit into another, but these processes depart in several ways from the conditions found in normal peripheral stumps or in autografts.

The break-up of myelin proceeds more slowly than usual, so that in some parts of a homograft of 25 days, especially at the centre, there may be fibres which have not yet broken up, or are in the process of doing so abnormally (Pl. 2, fig. 19). After breaking up, the myelin is found in fragments which are smaller than usual. The significance of this difference is not clear, probably it is connected with the fact that the invasion of macrophages is somewhat more marked in these grafts than in a normal peripheral stump. These

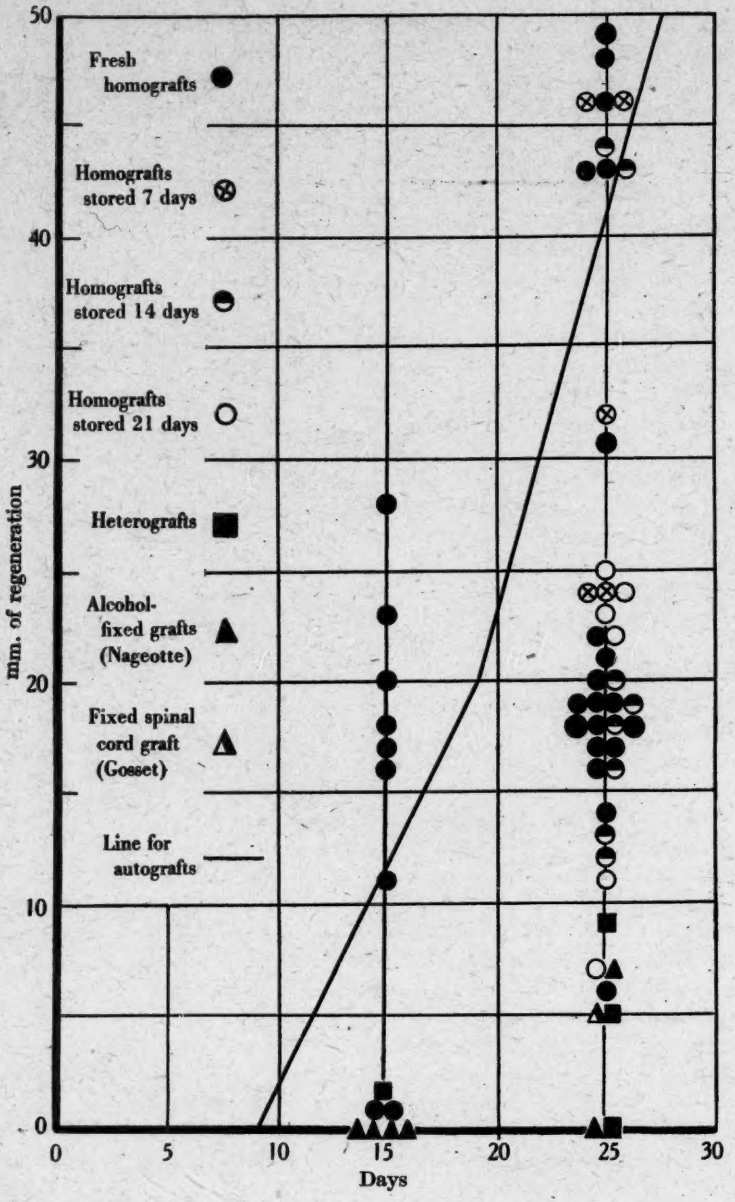


Fig. 2.

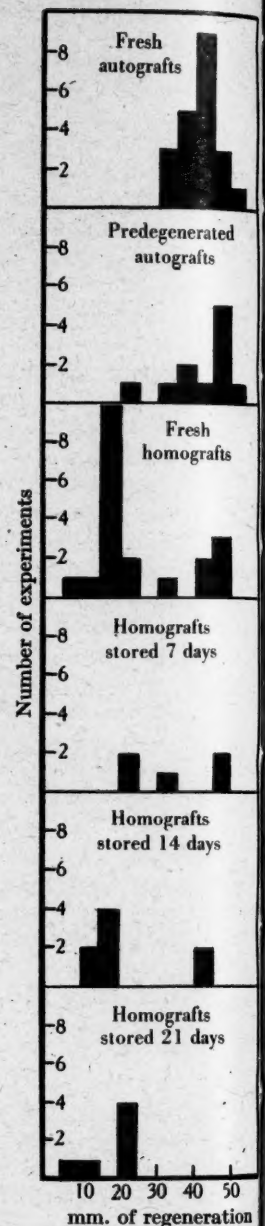


Fig. 3.

Text-fig. 2. Distances at which reflex responses were obtained after insertion into the peroneal nerve of grafts of various types. For comparison the line for fresh autografts is copied from Text-fig. 1.

Text-fig. 3. Histograms to show the frequency with which outgrowths of each distance were obtained, through various types of graft 25 days after insertion. Grouped by 5 mm. intervals.

abnormalities of degeneration result in, at best, a somewhat greater disturbance of the architecture of the nerve than is found in autografts.

In many cases the homografts produced much more extensive reactions. Large numbers of lymphocytes collected around the vessels in the graft and where host and donor tissues were in contact, especially at the junctions (Pl. 2, fig. 13). The reaction is not always equally severe. For instance, in the case shown in Pl. 2, fig. 12, it is minimal, but in other cases it may result in the filling of large parts of the graft with masses of small cells (Pl. 2, fig. 13). It must be remembered, however, that this study is limited to grafts 25 days old, and it is not possible to be certain of the fate of the tissues affected by such a reaction. Possibly the reaction may subside and leave the graft in a healthy condition. However, in some cases, after 25 days patches of necrosis were observed (Pl. 2, fig. 17) and these could hardly return to normal.

Certainly the Schwann cells of a homograft are able to multiply, even when a considerable lymphocytic reaction is present, and they produce Schwann bands which may approach normal appearance in the less disturbed parts of the graft. New nerve fibres can certainly enter in large numbers (Pl. 3, fig. 22), though in some cases with severe reactions they are noticeably fewer than in autografts. Moreover, the new fibres begin to medullate in the upper part of the graft, even after 25 days (Pl. 2, fig. 18).

There are, therefore, good reasons for supposing that the tissues of a fresh homograft in the rabbit can survive and become incorporated with those of the host nerve. In some cases this is accomplished with a small reaction, producing a condition approaching that of an autograft, though in other cases the state of affairs may be less favourable.

Homografts stored in Ringer's solution at 2° C. The practical advantages of using stored nerves from another animal (or man) would be considerable and we have made preliminary investigation of the changes occurring under various conditions of storage.

In an initial operation, pieces of tibial nerve were taken aseptically from rabbits, fastened by means of silk to small glass capillary supports without stretching the nerve, and placed in Petri dishes on pieces of Ringer-soaked gauze. These dishes were kept in a refrigerator at about 2–4° C. After periods of storage of 7, 14, or 21 days, 2 cm. pieces of stored nerve were used to fill gaps in the peroneal nerves of other rabbits. After 25 days' incubation in the host the distance of fibre outgrowth was then estimated as usual, and the grafts examined histologically. This simple technique proved moderately satisfactory, but two grafts out of twenty-four became infected.

After 25 days in the host these stored homografts showed differences in appearance from fresh homografts. Stored grafts were rarely so swollen or so strongly ensheathed as fresh homografts, and adhesions between the graft and its bed were either slight or absent. In addition they were always shrunken in length (average length of graft at 25 days = 16·1 mm.).

Between the grafts stored for 7, 14, or 21 days there were no striking

macroscopic differences after 25 days in the host. Grafts stored for all three periods varied in appearance from that of normal nerve to a pale greyish yellow, and they always possessed a thin collaterally vascularized sheath. The only consistent difference was of vascularization, the grafts stored for 7 days being as a rule better vascularized than those at 14 or 21 days.

Text-figs. 2 and 3 show the distances reached at 25 days by sensory fibres growing through stored grafts. The fibres have grown on the average farthest through grafts stored for 7 days (average distance of outgrowth = 34·5 mm.). Nevertheless, two grafts stored for 14 days gave distances of outgrowth of 46 mm., comparable not only with similar values obtained from 7-day stored grafts, but with the best results of fresh homografts and autografts. The average distances of outgrowth after 14 and 21 days' storage are respectively 24 and 18·6 mm.

In so far as averages of such scattered data have any meaning they show that fibres grow further through the grafts stored for 7 days than through fresh homografts. This is also suggested by three cases in which grafts stored for 7 or 14 days were controlled by fresh homografts on the opposite side of the same animal. In all, the fibres had grown as far or farther in the stored grafts. Of five cases in which the grafts were stored for 21 days and similarly controlled, three showed greater distances in the stored, two in the fresh homograft.

Histology of Ringer-stored homografts. Break-up of the myelin has proceeded very completely in grafts stored for all the three periods. In none of these stored grafts were there to be seen any unbroken fibres such as were found in some homografts (Pl. 3, fig. 25). Moreover, after the myelin has become broken up it is removed from these grafts by the very great invasion of macrophages which takes place, although the removal is less rapid than in a normal peripheral stump. The graft becomes filled with rows of 'foam' cells in active phagocytosis, so that the internal architecture of the nerve may become considerably upset (Pl. 3, figs. 23 and 24). Small patches of necrosis were seen in one case.

The lymphocytic reaction which is so marked a feature in fresh homografts still appears, though reduced, in those grafts which have been stored for 7 days (Pl. 2, fig. 14). But in those stored for 14 or 21 days it is at most very slight; there may be small patches of cells here and there, but there is no general reaction wherever the graft touches the host tissues. As a result, the junctions made by these stored grafts are very good, being made with a minimum of reaction on the part of the host and the graft, so that the result appears very similar to a simple suture or an autograft (Pl. 2, figs. 15, 16; Pl. 3, fig. 20).

Cells which appear to be live Schwann cells were found in the grafts after all three periods of storage and 25 days in the host (Pl. 3, fig. 21). It is not impossible that these cells have invaded the graft from the host nerve, but as they are found in all parts of the graft at 25 days, there is every likelihood

that they are cells which have survived the time of storage. They are not so numerous as the Schwann cells in a normal peripheral stump or an autograft, and, although many of them have a normal appearance, others are broader than usual, and often irregular in shape.

Thus even after storage for 21 days, and incubation in the host for 25 days, the structure of the graft is to some extent preserved. Many of the collagenous tubes remain intact and are tenanted by Schwann cells. Into these tubes new fibres can be seen to penetrate in considerable numbers, though of course they are fewer than in an autograft. A curious feature is that the growing axons are abnormally thick (compare, for instance, fig. 23 with figs. 2, 6, or 22). After 25 days the fibres begin to acquire myelin sheaths (Pl. 3, fig. 25), a sign that live Schwann cells are present in the graft.

In one case a 14-day stored graft was studied after 73 days' incubation in the host. It had the appearance of normal nerve. The junctions had been made with a minimum of criss-crossing, and, although the graft still contained some few macrophages, it was tenanted by large numbers of Schwann cells, and traversed by many new myelinated fibres.

There seems every reason to suppose, therefore, that storage of grafts in this way is possible, and may be even advantageous in that it reduces the lymphocytic reaction. Some at least of the cells of the graft can survive, and it seems likely that they would provide a basis for some functional recovery. It must not be forgotten, however, that the graft becomes filled with an excessive number of macrophages, and that the number of new fibres entering it, though considerable, is certainly less than in an autograft. Until means can be found to reduce these disadvantages, stored homografts would, *ceteris paribus*, be less likely to be successful than autografts.

FRESH HETEROGRAFTS

These grafts were 2 cm. long and of three kinds:

(1) Pieces of the whole sciatic, or the tibial only, of rats (four grafts).

(2) Pieces of the peroneal nerve of dogs (one graft).

These were used to fill gaps in the peroneal of the rabbit.

(3) In one case, 5 strands of rabbit tibial were stuck together with plasma to make a cable graft 2·4 cm. long, which was inserted into the sciatic of a dog.

At 15 and 25 days both of the first two kinds of graft had the same appearance. They were bright yellow, shrunken in length (average length of grafts at 25 days = 15·75 mm.), and never vascularized. They were enclosed in a tough fibrous sheath which was strongly adherent to the surrounding tissues. The grafts were never properly united with the peroneal nerve at the ends, and felt hard or 'doughy' when pinched.

The distances of sensory fibre outgrowth were small. At 15 days there were no fibres across the proximal scar, while at 25 days the fibres had occasionally grown a short distance (under 10 mm.) into the graft (Text-fig. 2).

Histology of fresh heterografts. Histological examination showed that both dog and rat nerve transplanted into the rabbit peroneal set up a very marked and unfavourable reaction wherever the tissues of the two animals were in contact, that is to say, along the sides of the graft, and particularly at the ends. At these points there were vast collections of white cells, including lymphocytes, oxyphils, and some macrophages. The reaction was so great as to cause considerable swelling of the ends of the nerves, and it entirely prevented the proper 'healing' of the graft into the host nerve, so that the junction of the tissues of the two animals shows a clear line of separation even after 25 days (Pl. 3, figs. 26, 27).

Within the graft itself the reaction was much less marked. There was less invasion by histiocytes than even in a normal peripheral stump. Moreover, the Schwann cells had not undergone proliferation (Pl. 3, fig. 28), and there had been no breaking up of either the axon or the myelin (Pl. 3, fig. 29).

It may be said, therefore, that these heterografts fail to degenerate, or at best do so to a minimal extent and in an abnormal manner. This would constitute a strong contra-indication to the use of heterografts in man even apart from the cellular reactions at the ends of the graft. Under these conditions it is not surprising to find that only a very few fibres penetrate the graft, and these only for short distances.

In the one case in which rabbit nerve was grafted into a dog the results were rather different. A very extensive cellular reaction was set up, within as well as around the graft. The outlines of the latter were difficult to trace, and the tissues were penetrated by a vast number of cells, including many macrophages, polymorphs, and lymphocytes (Pl. 4, fig. 30). The first-named were especially active (in contrast to the heterografts placed in the rabbit), and had gone very far, after 25 days, towards complete removal of the graft. Such traces of structure of the latter as remained, however, showed that the Schwann cells had undergone proliferation, and along the bands so provided fibres had penetrated for a distance of 10 mm. However, they were few in number, and the whole state of the graft was not such as to provide, in itself, a good union between the stumps. It seems not impossible, however, that fibres would ultimately have reached the peripheral stump, and perhaps even have produced some functional return, though less than could be obtained with an autograft.

Blondin (1928) found functional recovery after grafts of rabbit on dog sciatics. Cajal (1928), and Eden (1919), both condemn the use of heterografts, although Huber (1895) claimed some success for them experimentally, although not so much as for fresh autografts or homografts. Both Blondin (1928) and Huber (1895) used dogs as their experimental animals. It is probable that in their experiments the grafts were largely removed, and union of fibres with their end-organs occurred by growth through the replacing tissues.

DEAD HOMOGRAFTS PRESERVED IN ALCOHOL

These grafts were prepared according to the method of Nageotte (1917*c*). Grafts were taken from an animal and fixed in 90 % alcohol. At the end of 24 hr. they were transferred to 50 % alcohol and kept there until required for use. 24 hr. before insertion they were taken from alcohol and washed in several changes of Ringer's solution to remove the alcohol. The grafts were always 2 cm. pieces of rabbit tibial nerve, and were inserted into the rabbit peroneal nerve in the way already described.

In contrast to fresh homografts and autografts, dead grafts examined at 15 days were never properly united with the host peroneal. They were bright yellow in appearance, felt hard when pinched, and were never vascularized. They were always enclosed in a thick, collaterally vascularized capsule, which was strongly adherent to the surrounding tissues.

At 25 days the same features were present and were even more apparent. The junctions appeared to be invaded laterally by connective tissue.

In one animal opened at 61 days the graft was found to be represented by a small nodule of bright yellow tissue not attached to either stump. It lay laterally in a thick strand of greyish tissue connecting the stumps, which had the appearance of a tissue proliferation of double origin from the central and peripheral stumps of the peroneal, similar to the spontaneous junctions first noted by Kirk & Lewis (1917).

At 15 and 25 days outgrowing fibres were only rarely found to have grown across the proximal junction. Text-fig. 2 shows that the only growth obtained was in one case where at 25 days pinching showed that the fibres had apparently grown 7 mm. into the graft; but the histology showed these fibres to be growing down outside the graft.

Histology of alcohol-preserved homografts. After 15 days the graft is beginning to be invaded by macrophages. This process goes on especially at the ends, and at this stage invasion of the graft from the sides is slight. The reaction produced is not a very violent one, but is limited to a gradual macrophage invasion, removing the grafted tissues. *The nerve fibres certainly do not penetrate the original tissues of the graft* but advance with the macrophages, and at the boundary of the digested and undigested tissue form a plexus (Pl. 4, fig. 31), many being diverted laterally and showing large end-bulbs and other irregular formations. Other strands of outgrowing fibres progress down outside the graft.

By 25 days these processes have gone further. Macrophages are now attacking the sides of the graft. Short portions at the ends have already been completely removed, leaving a mass of macrophages with some fibroblasts. Through this mass Schwann cells and nerve fibres grow out from the central stump, and Schwann cells alone from the peripheral. In this way new nerve is being built as fast as the old is removed, but of course with considerable criss-crossing at every level (Pl. 4, fig. 32).

In the graft examined at 61 days these processes had been carried much further. The original tissues of the graft had been almost completely destroyed by macrophages (Pl. 4, fig. 34), and were being actively replaced by those of the host, so that at the edges of the graft, and especially at the ends, stretches of fibroblasts and Schwann cells, running in longitudinal directions, had appeared (Pl. 4, figs. 33, 35). In this way a tissue resembling normal nerve was being rebuilt, and in places, particularly at the edges, consisted of dense bundles carrying considerable numbers of nerve fibres. The centre of the graft had been less fully replaced, and carried few fibres, though it is possible that it would have come to carry more later. There is thus no doubt that the graft has served, in a sense, to effect union of the stumps, partly by providing a bridge *outside* which fibres can grow, and partly by actual replacement of a region of the graft by new nerve. So far as these cases show, however, there is *no retention of the tissues of the original graft*, which are replaced by a mass of macrophages, within which new and longitudinally orientated tissues appear.

Thus it may be that alcohol-fixed homografts act as a guide for proliferations from both stumps, which grow out as the graft is gradually removed, and eventually meet and become innervated. Huber (1920) reported functional recovery with these grafts, but he only studied late regenerates, by which time the actual graft could easily have been removed and replaced. Nageotte (1917*b*, *c*) also studied alcohol-fixed grafts histologically, and claims to have found them innervated in the dog at 56 days. It is probable, however, that little of the graft is left at 56 days, and, as Nageotte seems only to have studied transverse sections of his grafts, he may have observed an innervated proliferation. The present results support those of Cajal (1928). The actual graft in alcohol-fixed grafts is not innervated.

If the success found with alcohol-fixed grafts in animals is due to their action as guides for proliferations growing out from the stumps of the host nerve, we have an explanation for their lack of clinical success (see Laduron & Christophe, 1938). In a wound in man involving severance of a nerve trunk, the considerable scarring of the bed in which the graft would lie would not be likely to favour the outgrowth of proliferations replacing the graft. In any case the amount of criss-crossing and loss of fibres would be such as to prevent the achievement of any satisfactory return of the complex functions of a mixed nerve such as the median (see below, p. 161).

DEAD HETEROGRAFTS OF ALCOHOL-PRESERVED SPINAL CORD

One of these grafts was prepared and inserted according to the method of Gosset & Bertrand (1938). The spinal cord of a rat was fixed in situ in a mixture of 20 parts of neutral formaldehyde with 80 parts of water. Next day it was removed from the vertebrae and placed in 50 % alcohol for a week. 24 hr. before insertion a 2 cm. length was taken and washed in several changes

of sterile Ringer's solution.[1] It was then taken and inserted into a gap in the peroneal nerve of a rabbit.

At 25 days this graft was bright yellow, shrunken, and not vascularized. It felt 'doughy' to pinching, which showed a distance of outgrowth of 5 mm. (Text-fig. 2), and was enclosed in a thick capsule, strongly adherent to the surrounding muscle.

Histology of alcohol-preserved spinal cord graft. Histological examination of the grafted piece of spinal cord recovered after 25 days showed the least favourable reaction of all the types of graft examined. The whole graft was enclosed in a fibrous capsule, within which it was being vigorously attacked by giant cells (Pl. 4, figs. 36, 38). These form a complete row all round the graft, and would presumably ultimately remove the whole of it. There has been no union of the graft with either central or peripheral stump, and there seems no likelihood that such a union would have taken place if the graft had been left for a longer period. Outgrowth had taken place from both stumps *alongside* the graft (Pl. 4, figs. 36, 37) and it is possible that in time a union would be formed in this way *outside* the graft. This union might make a basis for some functional recovery in animals, and perhaps even in man, but, as explained on p. 161, such recovery could at best only be partial on account of the small number of fibres which would return to appropriate end stations after the passage of such a long gap in irregular bundles. In any case the graft is not serving to unite the stumps by virtue of its nature as a piece of spinal cord, but merely as a support along which outgrowth from the stumps may take place. As shown above, this is part of the function of an alcohol-fixed homograft, but the reaction to the two types of graft is not similar—at least in the rabbit. The bed of macrophages left in place of a piece of alcohol-fixed nerve provides a possible basis for the formation of new nervous tissue, whereas the complete encapsulation of the piece of spinal cord would seem to prevent its penetration by nerve fibres even after removal of the grafted tissue. It would require investigation of further cases to establish the reason for this difference in behaviour of the host towards pieces of material which are both treated as foreign bodies, in the sense that no part of them is incorporated into the host.

Gosset & Bertrand (1938) claimed success for these grafts in four dogs, as judged by the recovery of a normal chronaxie in the paralysed muscles, but recovery of sensation and function was not adequately studied. From the above experiment it seems unlikely that recovery could be anything but partial in the rabbit.

This unfavourable result contra-indicates the use of 'Gosset' grafts in man. Even if they do support Schwann bands growing beside them from the cut stumps, they are likely to fail in a wound for the same reason as alcohol-fixed homografts.

[1] Gosset & Bertrand used serum for this washing. It is unlikely that the change to Ringer influenced the final result.

DISCUSSION

In the application to man of the results of these animal experiments it is necessary to consider possible differences in (1) tissue reactions, (2) size, and (3) complexity of functional requirements. As regards the first, the reactions produced by any type of graft are unlikely to be identical in man and rabbit, but it seems probable that autografts can survive, degenerate, and become re-innervated in both.[1] There is ample evidence (Foerster, 1929; Bunnell & Boyes, 1939; Ballance & Duel, 1932*b*) to show that under some conditions functional recovery can take place after autografting in man. No full histological study has been made of the fate of human autografts, though those which have been recovered have been reported as converted into scar tissue (Stopford, 1920; Platt, 1921). It may be that this is the fate of a graft placed under unfavourable conditions. But, as well as the present work, the clinical experience of workers as varied as Foerster, Duel & Ballance, and Bunnell & Boyes shows that autografts can survive (see also footnote, p. 146).

The factor of size has never been carefully studied. The grafts in the present series were all less than 4 mm. in diameter. Those in the facial canal and the extremities were also small, while those of Foerster were cable grafts. Bielschowsky & Unger (1917) claim that in dog grafts the centre of the graft becomes necrotic and does not carry any new fibres, unless it becomes very quickly vascularized. Maccabruni (1911) found that when the graft was thick there was a necrotic central zone, and the degeneration was atypical, while this was not true of small, thin, grafts. Bunnell & Boyes (1939), in the cat, found fibres more abundant in the periphery of their grafts, while there was necrosis at the centre.

There seems no reason to suppose that long grafts would be any less successful than short. The rate of growth in autografts is little less than in a normal peripheral stump, and in the three experiments of the present series in which grafts of 5 or 6 cm. were taken, fibres had proceeded at this rate along

[1] Through the co-operation of Miss E. Bülbring of the Department of Pharmacology we were able to show that similar changes occur in the early stages after grafting in dogs. A 4 cm. length of tibial nerve was inserted with plasma into a gap in the peroneal nerve of the same dog. When examined 21 days later this autograft had the appearance of a normal peripheral stump. Sections showed that degeneration, removal of myelin, and Schwann cell proliferation had proceeded almost normally. There were rather more macrophages in the graft than in the peripheral stump, and in a few places there were collections of polymorphs and lymphocytes, but although the graft was 5 mm. thick there was no sign of necrosis at its centre. Nerve fibres were growing down the graft.

In three other dogs homografts of 35, 45 and 28 mm. were inserted into the sciatic. These grafts, when examined 12, 25 and 29 days later, appeared much swollen. Sections showed extensive collections of lymphocytes and polymorphs within and around the grafts. Many macrophages were present, but the break-up and removal of axons and myelin had proceeded much less far than in the peripheral stumps, or in the autograft described above. Moreover, there had been very little proliferation of the Schwann cells. Some nerve fibres were growing down the grafts.

We are most grateful to Miss Bülbring for her help in showing, by these experiments, that in dogs, as in rabbits, autografts degenerate almost normally, but that there is some abnormality in homografts.

the whole graft. Moreover, as shown on p. 148, there is no reason to suppose that the lower junction is necessarily badly made in the absence of nerve fibres.

The case of homografts is less easy to discuss because the possibility of differences between man and rabbit is considerable. Only experiment can show whether the somewhat unfavourable reactions observed in the rabbit are found also in man, even between members of the same blood group. These reactions appear to be due to some substance in the graft, which is removed or destroyed during storage, and this, or some other method of treatment, may be found valuable in man.

The fact that no proper degeneration occurs in heterografts confirms the observations of Merzbacher (1905), and shows very clearly that the processes of 'degeneration' are not a mere passive breaking up, even in the case of myelin, but an active process produced by the cells of the nerve.

Factors affecting the quality of recovery. A nerve graft in man is only valuable if it conducts to the correct part of the periphery an adequate number of sensory and motor nerve fibres. Since the number of different types of fibre in a nerve is generally greater in man than in animals and the functions to be repaired correspondingly more delicate, this requirement restricts the possibilities of successful human recovery. It is therefore essential in man to use only those means of bridging gaps which give every possible opportunity to the new fibres. For instance, alcohol-fixed nerves, after being destroyed by macrophages, may be replaced by a new outgrowth of nervous tissue (p. 157). It is even possible that by this means a functional recovery can be obtained in animals (Nageotte, 1917*c*; Huber, 1920). But the criss-crossing which is bound to occur repeatedly as the new fibres advance against the barrier of unremoved graft (see Pl. 4, fig. 32) must reduce the number of fibres which return close to their original endings. In a simple nerve whose fibres are of a few types this may not matter, but it may well prevent all useful recovery of delicate functions such as those of the median nerve in man. Again, such procedures as the fixed spinal cord graft in the present series encourage, or at least allow, outgrowth from central and peripheral stumps by which union can be effected (see Young *et al.* 1940). But, for the above reason, recovery produced in this way is not likely to be complete in man.

It is important finally to consider whether the condition in autografts is likely in any similar way to prejudice the quality of recovery. Certainly a great number of fibres can penetrate an autograft. No careful counts have yet been possible, but the density does not appear to be greatly less than in a normal peripheral stump. However, a possible adverse factor in autografts is the disturbance of the structure of the nerve which is produced by the delay in the removal of myelin remains. Comparison of figs. 3 and 7 in Pl. 1 suggests that this might be an important effect. However, the disturbance is usually less extreme than that shown in fig. 7.

The interposition of an extra point of junction must also be a factor militating against the success of any graft. A good deal of criss-crossing is

inevitable, although, as has been shown (p. 148), there is no reason to suppose that fibrosis need occur at a well-made junction.

These then are factors which must tend to reduce the number of fibres returning to 'correct' endings through a graft. It would be a mistake to give them undue emphasis as contra-indications for grafts until it is known to what extent the superfluity of fibres produced during regeneration allows for loss during transit. It may be that within reasonable limits, and assuming approximately correct apposition of bundles, re-connection of paths is always a statistical accident, so that a small reduction of numbers does not greatly affect the result. The impossibility of reaching any decision on the point at present emphasizes the importance of devising in the future a method for assessing the *quality* of recovery both in man and in animal experiments.

SUMMARY

1. Various types of nerve graft were inserted into the peroneal nerve of the rabbit. After 15 or 25 days, and in some cases longer, examination was made of the distance of outgrowth of pain fibres, and the histological changes in the graft.

2. The Schwann cells of an autograft survive and multiply, and the myelin breaks up and is removed, though rather slowly. New pain fibres grow through the graft at the rate of 2·0 mm./day (cf. 3·5 mm./day in a normal peripheral stump). After 25 days fibres are already medullating in an autograft.

3. Fibres crossed the lower junction of autografts with a maximum delay of 1·7 days. Histologically it can be seen that the junction is made by Schwann cells and is not dependent upon the presence of nerve fibres. This is so even for grafts 5 cm. in length. There is, however, a tendency for grafts to shorten, which may lead to bad junctions. Apart from this there is no reason to resort to resuturing a lower junction.

4. Nerve fibres do not grow faster through pre-degenerated than through fresh autografts. Any advantage of pre-degeneration must therefore be in the consistency of the graft.

5. Homografts often set up a considerable lymphocytic reaction which may lead to partial necrosis. Proliferation of Schwann nuclei and myelin break-up proceed in the graft, though the break-up may be abnormal. The distances reached by new fibres are more variable and smaller than in autografts. However, the new fibres medullate in the graft. In the favourable cases homografts therefore provide a possible basis for recovery.

6. In homografts stored for 7–21 days in Ringer at 2° C. the lymphocytic reaction is reduced or absent. There is a great invasion by macrophages, but Schwann cells survive and proliferate, though not normally, and myelin break-up proceeds. New fibres grow into the graft to distances which are

variable, but sometimes greater than in control fresh homografts. However, the fibres are abnormally thick and rather few in number.

7. Heterografts of dog or rat in rabbit set up a vast reaction at their edges, and do not degenerate. There is no Schwann proliferation or myelin break-up, and few new fibres enter the graft. A graft of rabbit into dog produced a very great reaction by which most of the graft was removed.

8. Pieces of nerve fixed in alcohol and used as suggested by Nageotte are removed by macrophages. New nerve fibres do not enter the actual transplanted tissue, though they may grow well into the bed of macrophages and fibroblasts which replaces it, and in this way reach the peripheral stump. Reunion by such a method involves at best much criss-crossing.

9. An alcohol-preserved piece of formol-fixed rat spinal cord (Gosset) was encapsulated and attacked by giant cells. New outgrowths passed around it but not within it.

10. This study of the early fate of grafts shows that in the rabbit autografted nervous tissue can survive, with degeneration and proliferation of Schwann cells, making a good basis for recovery. Homografts can also do so, but in some cases set up reactions. These reactions are reduced by previous storage of the graft. Heterografts set up a very great reaction and do not degenerate. They and alcohol-fixed grafts only provide for reunion of stumps after destruction and replacement by host tissues and are therefore unlikely to provide for a successful recovery in man.

This work has been made possible by the assistance of the Rockefeller Foundation, and we are deeply grateful to the Foundation and its Officers for their continued support.

We wish to thank Dr P. B. Medawar for assistance with statistical work and Prof. E. S. Goodrich, F.R.S., for reading the manuscript of this paper.

REFERENCES

Ballance, C. & Duel, A. B. (1932*a*). *Arch. Otorhinolaryngol.* **15**, 1.
—— —— (1932*b*). *Arch. Otorhinolaryngol.* **16**, 767.
Bentley, F. H. & Hill, M. (1936). *Brit. J. Surg.* **24**, 368.
—— (1940). *Brit. med. J.* **2**, 352.
Bethe, A. (1916). *Dtsch. med. Wschr.* **2**, 1311.
Bielschowsky, M. & Unger, E. (1917). *J. Psychol. Neurol.* **22**, 267.
Blondin, J. (1928). Contribution à l'étude des greffes des nerfs. Thèse de Paris.
Bunnell, S. & Boyes, J. H. (1939). *Amer. J. Surg.* **44**, 64.
Cajal, Ramon y (1928). *Degeneration and Regeneration of the Nervous System.* Oxford.
Collier, J. (1940). *Lancet*, **2**, 91.
Davis, L. & Cleveland, D. A. (1934). *Ann. Surg.* **99**, 271.
Duel, A. B. (1933). *Surg. Gynaec. Obstet.* **56**, 382.
Eden, E. (1919). *Arch. klin. Chir.* **112**, 471.
Foerster, O. (1929). *Handbuch der Neurologie*, Teil II, Abs. 3. Berlin.

Gosset, A. & Bertrand, I. (1938). *J. Chir., Paris*, **51**, 484.
Huber, G. C. (1895). *J. Morphol.* **11**, 629.
—— (1919). *Arch. Neurol. Psychiat.* **2**, 466.
—— (1920). *Surg. Gynaec. Obstet.* **30**, 464.
Kilvington, B. (1908). *Brit. med. J.* **1**, 1414.
Kirk, E. G. & Lewis, D. D. (1917). *John Hopk. Hosp. Bull.* **28**, 71.
Laduron, E. & Christophe, L. (1938). *Rapp. Ann. Soc. Belg. Chir.*, Séances extraord., p. 122.
Maccabruni, F. (1911). *Folia Neurobiol.* **5**, 598.
Medical Research Council, London (1920). *Report* no. 54.
Merzbacher, L. (1905). *Neurol. Zbl.* **24**, 150.
Nageotte, J. (1917*a*). *C.R. Soc. Biol., Paris*, p. 459.
—— (1917*b*). *C.R. Soc. Biol., Paris*, p. 889.
—— (1917*c*). *C.R. Soc. Biol., Paris*, p. 925.
Phillipeaux, J. M. & Vulpian, A. (1870). *Arch. Physiol. norm. path.* **3**, 618.
Platt, H. (1919). *Brit. J. Surg.* **7**, 384.
—— (1921). *The Surgery of the Peripheral Nerve Injuries of Warfare.* Bristol Hunterian Lecture.
Sargent, P. (1920). *Brit. med. J.* **2**, 465.
Stopford, J. S. B. (1920). *Lancet*, **2**, 1296.
Tello, F. (1915). *Progresos de la clin.* March.
Young, J. Z., Holmes, W. & Sanders, F. K. (1940). *Lancet*, **2**, 128.
Young, J. Z. & Medawar, P. B. (1940). *Lancet*, **2**, 126.

EXPLANATION OF PLATES 1–4

Except where stated the figures are of material removed 25 days after the initial suture or graft in the rabbit.

Plate 1

Fig. 1. Simple junction with plasma of peroneal into tibial nerve, made 25 days previously. The central stump (peroneal) lies to the right. Bodian's stain. (82e.4.1.)

Fig. 2. Portion of tibial nerve 1 cm. below a simple plasma suture made 25 days previously. Note numerous Schwann nuclei, ingrowing axons, macrophages, a narrow blood-vessel. Bodian's method. (185c.5.9.)

Fig. 3. Portion of peroneal nerve 6 mm. below the lower junction of a homograft made 25 days previously. More or less regular arrangement of Schwann bands, but with some interference by myelin remains. No cellular reaction around the blood vessel on the right. Bodian and Masson. (316d.8.2.)

Fig. 4. Portion of peroneal nerve 1 cm. below the lower junction of a homograft to show myelin remains. Flemming's fluid, Weigert stain. (198b.10.8.)

Fig. 5. Upper junction of autograft. The graft lies to the right. Bodian and Masson. (277d.4.13.)

Fig. 6. Portion of an autograft, for comparison with fig. 2. There are many Schwann nuclei, nerve fibres and macrophages. Note swollen blood vessel. Bodian. (198d.5.13.)

Fig. 7. Portion of an autograft in which the myelin remains considerably disturb the architecture of the nerve. There is also some lymphocyte reaction around the blood vessels. Bodian. (71h.11.3.)

Fig. 8. Portion of an autograft to show normal break-up of myelin but somewhat delayed removal of remains (compare fig. 4). The new fibres in the graft are already becoming medullated. Flemming's fluid, Weigert. (126e.9.3.)

Plate 2

Fig. 9. Lower junction of an autograft inserted 15 days previously. The graft is to the left. A smooth union has been made although no nerve fibres have yet arrived. Bodian-Masson. (59e.12.8.)

Fig. 10. Lower junction of an autograft inserted 30 days previously, showing Schwann bands running directly from the graft (on right) to the host nerve. Masson's stain. (399a.1.1.)

PLATE 2 (*continued*)

Fig. 11. Portion of autograft to show normal break-up of myelin and medullation of the new fibres which have entered the graft. Flemming-Weigert. (126c. 9. 2.)

Figs. 12–16 are of the upper junctions of various homografts. All have been taken at the same magnification and in all the graft occupies the lower portion of the picture.

Fig. 12. Homograft, showing a small amount of cellular reaction. Bodian. (316d. 10. 10.)

Fig. 13. Homograft showing a very extensive reaction around the graft. Bodian-Masson. (102d. 6. 12.)

Fig. 14. Homograft stored for 7 days in Ringer's solution at 2° C. before insertion. Small reaction. Bodian Masson. (376c. 14. 8.)

Fig. 15. Homograft stored for 14 days. Very little reaction. Bodian. (198a. 6. 2.)

Fig. 16. Homograft stored for 21 days. Very little reaction. Bodian. (398a. 6. 14.)

Fig. 17. Portion of homograft showing very extensive lymphocytic reaction and some necrosis. Bodian-Masson. (316d. 8. 2.)

Fig. 18. Portion of homograft near upper junction to show break-up of myelin and medullation of the new fibres invading the graft. The upper end is to the left and the first myelin is being deposited about the level of the middle of the photograph. Formol-Weigert. (233a. 7. 5.)

Fig. 19. Lower portion of the same graft as fig. 18, showing fibres whose sheaths have not become broken up even after 25 days.

PLATE 3

Fig. 20. Homograft stored for 7 days before insertion. Lower junction. The graft is at the upper end. There has been little reaction and the junction is very well made. Bodian. (385b. 1. 2.)

Fig. 21. Portion of homograft stored for 21 days before insertion, showing a single Schwann cell, which has presumably survived the period of storage, and several macrophages. Bodian-Masson. (398a. 6. 2.)

Fig. 22. Fresh homograft, showing lymphocytic reaction around a blood vessel. New nerve fibres have entered the graft. Bodian. (102d. 7.)

Fig. 23. Homograft stored for 14 days before insertion. Compare with fig. 22. There are no lymphocytes around the blood vessels but there is a considerable macrophage reaction. The ingrowing nerve fibres are abnormally thick. Bodian. (198a. 6. 2.)

Fig. 24. Homograft stored for 21 days before insertion. Compare with figs. 7, 3, 17. Note proliferation of Schwann nuclei, absence of lymphocytes, presence of numerous macrophages and some nerve fibres. Bodian. (397a. 5. 15.)

Fig. 25. Homograft stored for 14 days before insertion, showing normal break-up of myelin and some very thinly medullated new fibres. Flemming-Weigert. (198b. 10. 8.)

Fig. 26. Heterograft of dog nerve in rabbit. Upper junction, the graft is to the right. Note very great lymphocytic reaction and no proper union. Bodian-Masson. (273b. 13. 10.)

Fig. 27. Lower junction of the same heterograft as fig. 26; graft to the left.

Fig. 28. Portion of heterograft of rat into rabbit. Axons not broken up even after 25 days, no proliferation of Schwann nuclei, no macrophage or lymphocyte reaction. Bodian-Masson. (368a. 8. 9.)

Fig. 29. Heterograft of rat into rabbit. Upper end of graft stained osmium tetroxide to show myelin not broken up. (281c. 24. 4.)

PLATE 4

Fig. 30. Heterograft of rabbit in dog to show invasion by macrophages and complete destruction of graft structure except for isolated Schwann cells. Masson. (Dog 5a. 18. 3.)

Fig. 31. Alcohol-fixed (Nageotte) graft made 15 days previously; upper junction. The grafted material appears on the left. On the right can be seen new fibres growing from the central stump through a bed of macrophages which are attacking the graft. Bodian. (59b. 8. 7.)

Fig. 32. Alcohol-fixed (Nageotte) graft inserted 25 days previously, showing how new fibres grow out through the bed of macrophages which replaces the graft. The central stump appears above and from it proceed bundles of new fibres. The graft itself appears below but its upper and lateral portions have been removed already. Bodian. (60d. 10. 6.)

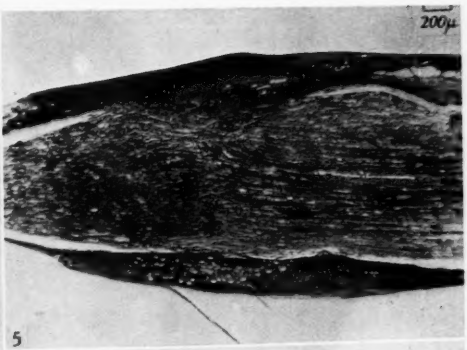
PLATE 4 (*continued*)

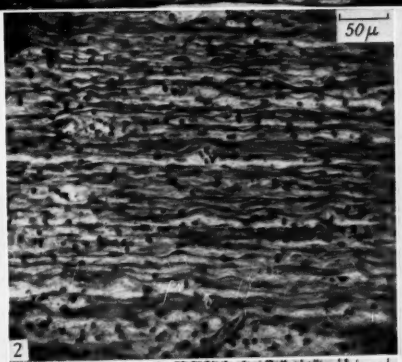
Figs. 33, 34 and 35 are portions of an alcohol-fixed (Nageotte) graft recovered 61 days after insertion. Fig. 34 shows the middle where the graft has been replaced by a mass of macrophages, in which a few collagen fibres are appearing. To the left of the graft are numerous fibrous bundles along which some Schwann cells have grown from the stumps. Fig. 35 is nearer to the central end and here the replacement of the macrophage bed by fibrous tissue is more advanced. In fig. 33 it is almost complete. Mallory. (137a.3.10.)

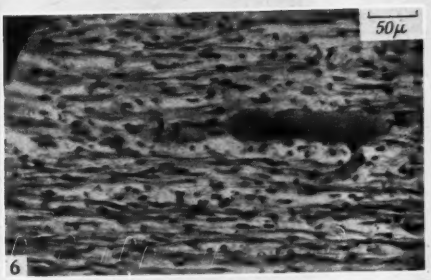
Fig. 36. Formol and alcohol-fixed spinal cord graft (Gosset). Upper junction. The graft has been enclosed in a fibrous capsule, and is itself being attacked by giant cells. From the central stump some outgrowth is proceeding alongside the graft. Bodian-Mallory. (223c.10.7.)

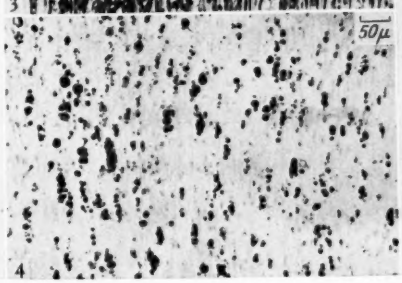
Fig. 37. Lower junction of the same graft as fig. 36. Some outgrowth from the peripheral stump alongside the graft.

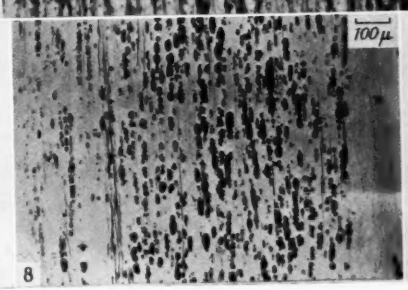
Fig. 38. Same graft as figs. 36 and 37, detail of giant cells attacking the graft. Bodian-Mallory. (223c.9.9.)



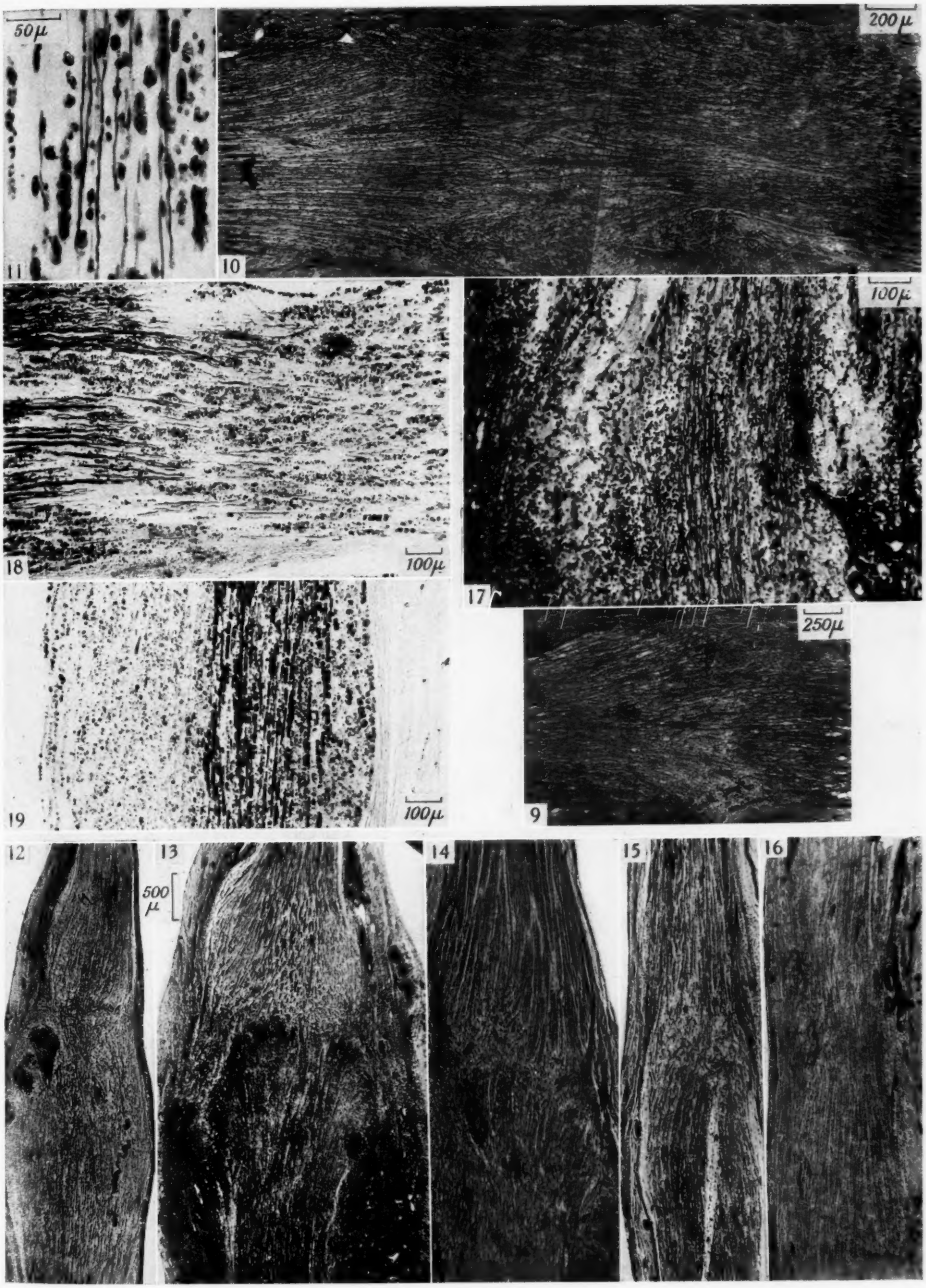






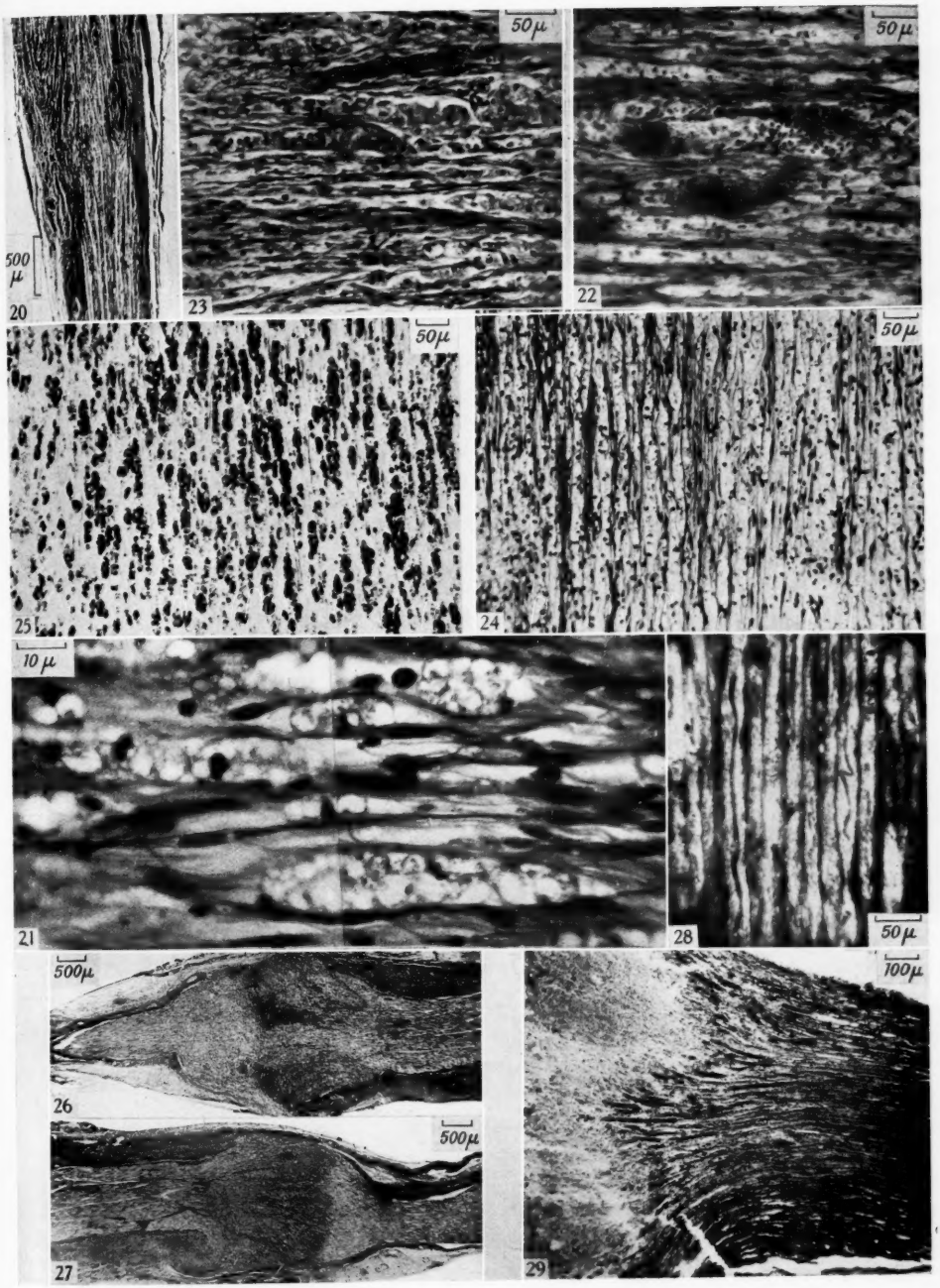


UNIV OF MICH.

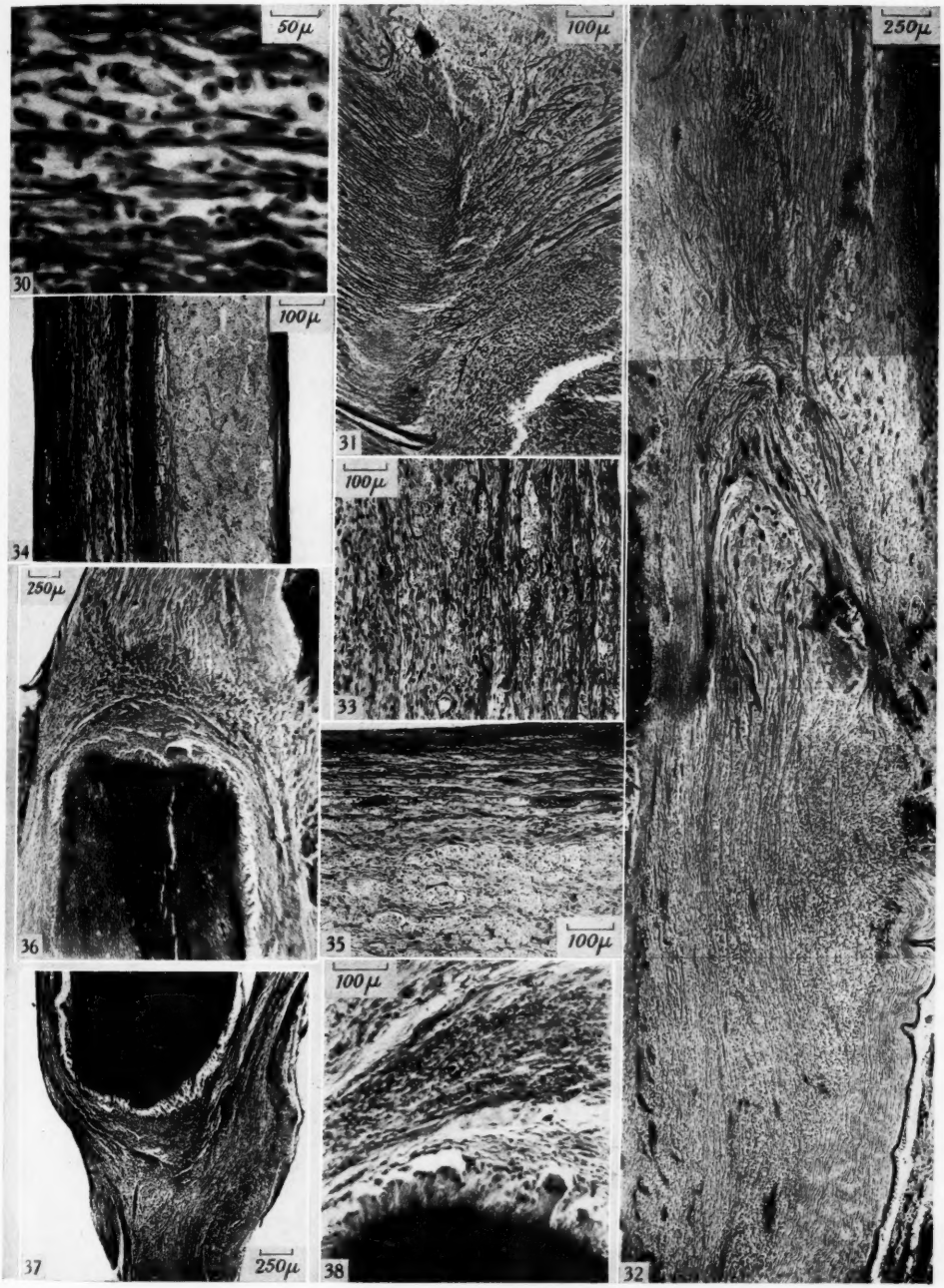


SANDERS AND YOUNG—NERVE GRAFTS

UNIV. OF MICH.


500 μ
20
50μ
23
50μ
22
50μ
25
50μ
24
10 μ
21
28
50μ
500μ
26
500μ
27
100μ
29

UNIV. OF MICH.



SANDERS AND YOUNG—NERVE GRAFTS

UNIV. OF MICH.

OBSERVATIONS ON JACOBSON'S ORGAN AND ITS INNERVATION IN *VIPERA BERUS*

By A. BELLAIRS

Interest has been aroused in the Jacobson's organ of snakes by the recent experimental work which suggests that this structure is one of the most important sense organs which these animals possess. It was originally suggested by Broman (1920) that the bifurcated tongue of snakes could be flickered into the organ of Jacobson or its duct, situated in the roof of the mouth, carrying odorous particles whose nature could thus be appreciated. There is now considerable evidence that this organ plays an important part in the feeding reactions of snakes and of some lizards (Kahmann, 1932; Noble & Kumpf, 1936; Wilde, 1938), in trailing the female in courtship (Noble, 1937), and a less important part in identifying other members of the same species in aggregation behaviour (Noble & Clausen, 1936).

At present it appears that no microscopic investigation of the innervation of Jacobson's organ, and the role (if any) of the nervus terminalis, has yet been made in snakes. The following observations are based upon the examination of a series of transverse sections through the head of an adult *Vipera berus*, cut at 15 μ, and stained according to the de Castro technique.

THE ORGAN OF JACOBSON

This organ is first seen in Amphibia, being present in Anura and Gymnophiones; it is interesting that the latter possess a peculiar retractile facial tentacle, which, according to Noble (1931), can be brought into contact with the epithelium in the neighbourhood of the organ of Jacobson, paralleling the function of the tongue tips in snakes. There is some doubt as to the identity of the food-taster of Urodeles, some regarding it as true vomeronasal epithelium which has migrated from the medial to the lateral side of the nasal cavity, while others, admitting its functional similarity, hold that it is derived from the existing epithelium of the lateral nasal sinus. According to Francis (1934), the latter view is now more generally held.

The organ is rudimentary in birds, crocodiles and some lizards, but is fairly well developed in other living reptiles, and relatively enormous in snakes. It is also present in most groups of mammals, and is most prominent in the monotreme *Ornithorhynchus*; in fact among the vertebrates it probably reaches its greatest development in this animal and in the snakes.

In our specimen of *Vipera berus*, Jacobson's organ, exclusive of its capsule, was about 1 mm. in antero-posterior length, and it can be seen as a black spot

beneath the epithelium of the roof of the mouth in preserved specimens. As in all snakes, the organ is completely closed off from the nasal cavity by its capsule, although, of course, it is continuous with it in the early stages of its development. The superior and lateral parts of the capsule are formed by the septomaxilla, while, medially and in front, the floor is formed by the paired

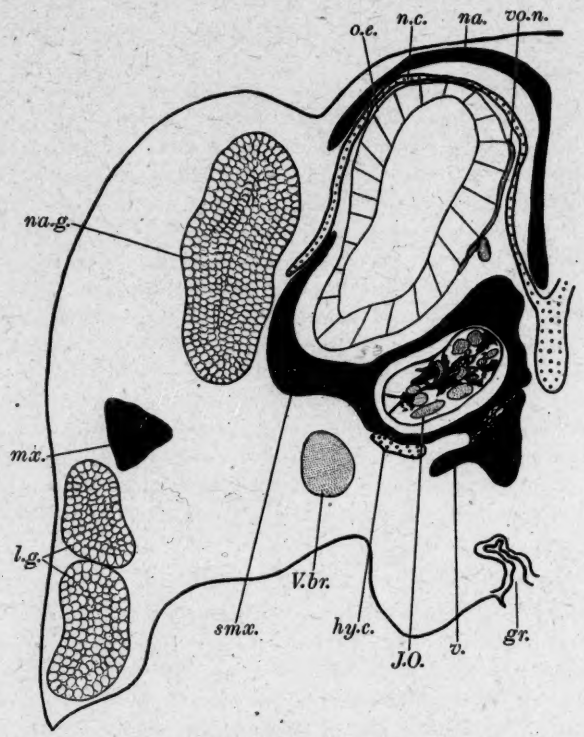


Fig. 1. Transverse section of the left side of the head through the anterior part of Jacobson's organ. *d.o.g.* duct of great orbital gland. *d.o.g.'* duct of great orbital gland (upper part). *d.o.J.* duct of Jacobson's organ. *ec.c.* ectochoanal cartilage. *gr.* paramedian groove into which duct of Jacobson's organ opens. *hy.c.* hypochoanal cartilage. *J.O.* Jacobson's organ. (In Fig. 1 the epithelium is cut transversely, and much pigment is seen among the epithelial cells). *l.g.* labial glands. *m.x.* maxilla. *na.* nasal bone. *na.g.* nasal gland. *n.c.* nasal capsule cartilage. *n.s.* nasal septum. *o.e.* olfactory epithelium. *p.f.* prefrontal. *smx.* septomaxilla. *v.* vomer. *vo.n.* vomero-nasal nerve. *V.br.* branch of V nerve.

vomer. Further back the bony floor is incomplete and the organ is supported by two cartilages. The more lateral of these is the ectochoanal cartilage (de Beer, 1937), while the medial, which projects into the lumen of the organ with its epithelial covering forming a concha-like prominence, represents the hypochoanal cartilage as described by Peyer (1912) for *Vipera aspis*, and Brock

(1929), for *Leptodeira hotamboia*. According to Broman (1920), pressure on the floor of the organ in feeding causes compression of the lumen which opens up again when the pressure is relaxed, sucking in fluid containing food particles from the mouth. The experiments of Wilde (1938) upon *Thamnophis* have shown, however, that the mere presence of stimulating particles upon the palate is sufficient to excite the organ, without the application of any pressure to its floor.

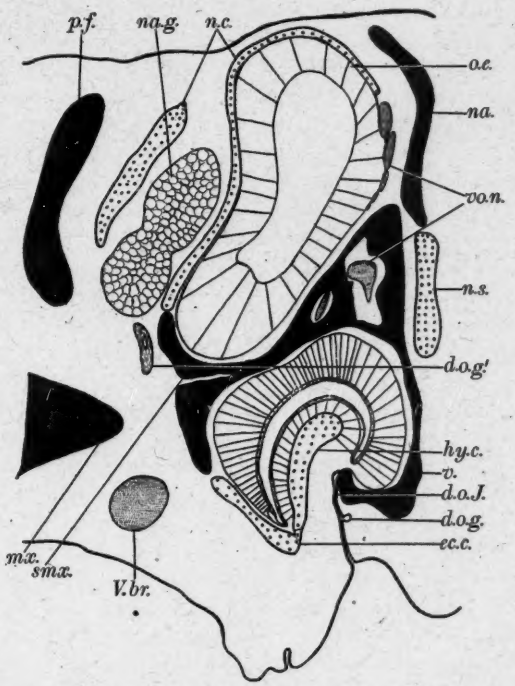


Fig. 2. The same as Fig. 1, a few sections farther back, showing the duct of the organ, and the duct of the great orbital gland opening into it. Lettering as in Fig. 1.

The paraseptal cartilages which participate in the capsule of the organ in lizards and *Sphenodon*, and form a complete cylindrical investment in *Ornithorhynchus*, are absent in snakes.

The organ of Jacobson opens to the mouth by a duct which passes almost directly downwards some distance in front of the posterior nasal opening. It opens immediately into a long groove which can be seen in preserved specimens on either side of the torus medialis; the groove itself extends forward for a millimetre or so in front of the opening of the duct, and is readily accessible to the tips of the tongue (Fig. 2). The duct itself is exceedingly narrow, with one or two blind diverticula opening into it as well as the duct of the orbital gland;

the walls were actually contiguous throughout most of its length, and the opening into the lumen of Jacobson's organ on the lateral side of the vomer was only patent in one section, so it would seem doubtful if the tips of the tongue could be inserted into the lumen of the organ itself.

According to Broman (1920), the duct in mammals never opens directly into the mouth, but communicates with it through the palatine duct.

The great orbital gland ('Harderian' gland)

No intrinsic glands associated with Jacobson's organ such as are present in mammals were found, although, according to Broman (1920), they appear in early embryos of *Natrix*, and later regress. The posterior part of the great orbital gland, which characteristically lies behind and outside the orbit, consists, in *Vipera*, of a small nodule lying under cover of the adductor mandibulae externus anterior muscle. It then passes forwards medial to the post-orbital bone and extends forwards beneath the optic nerve to reach the anterior boundary of the orbit, where the duct arises. This is at first greatly dilated, but later becomes narrower as it passes across the anterior margin of the prefrontal bone, and swings medially beneath the capsule of Jacobson's organ. It finally opens into the medial side of the lower part of the duct of the organ, and this terminal part is also continuous posteriorly with the nasal cavity.

This gland varies greatly in size and shape among different snakes, but its relations are essentially similar. In all species it reaches the orbit by passing medially to the post-orbital bone, when this is present, and then divides in most species into two finger-shaped lobes which embrace the optic nerve. The lower of these is the longer, and passes forward to the front of the orbit. In the genus *Vipera*, the gland is relatively small and the superior process hardly developed, but in some species, notably the egg-eaters *Dasypeltis* and *Elachistodon*, it is very large. Its size bears no relationship to the size of the eye, being fairly large in *Typhlops* (where the organ of Jacobson is said to be well developed) and other burrowing forms where the eyes are vestigial (Smith & Bellairs, unpublished observations).

The great orbital gland is frequently referred to as the Harderian gland, but it is doubtful whether it is homologous with the Harderian gland of other vertebrates where it is developed from the medial wall of the conjunctiva and discharges its secretion into the conjunctival sac, for the latter is absent in snakes. In lizards, a gland variously referred to as the lachrymal (Broman, 1920), or the Harderian (Schwartz-Karsten, 1937), is present, lying mainly in front of the orbit and communicating, according to Broman (1920), with the duct of Jacobson's organ, as in snakes, and according to Schwartz-Karsten (1937) with the conjunctival space. The latter suggests that the orbital gland of snakes has been derived from the lacertilian condition by posterior migration. Since this gland did not appear to discharge into the orbit in our *Vipera* it seems unlikely that it constitutes a true Harderian gland.

True lachrymal glands lying lateral to the lid-angle and discharging into the conjunctival sac have been described in the lizards, *Lacerta* and *Ophisops* by Schwartz-Karsten (1937), but no vestige of these could be found in *Vipera*.

The vomeronasal nerve

This nerve has been described by McCotter (1917) in *Rana catesbyana* and *Chrysemys marginata*, and its course and relations appear to be essentially similar in both forms, though the entire vomeronasal apparatus is relatively better developed in reptiles.

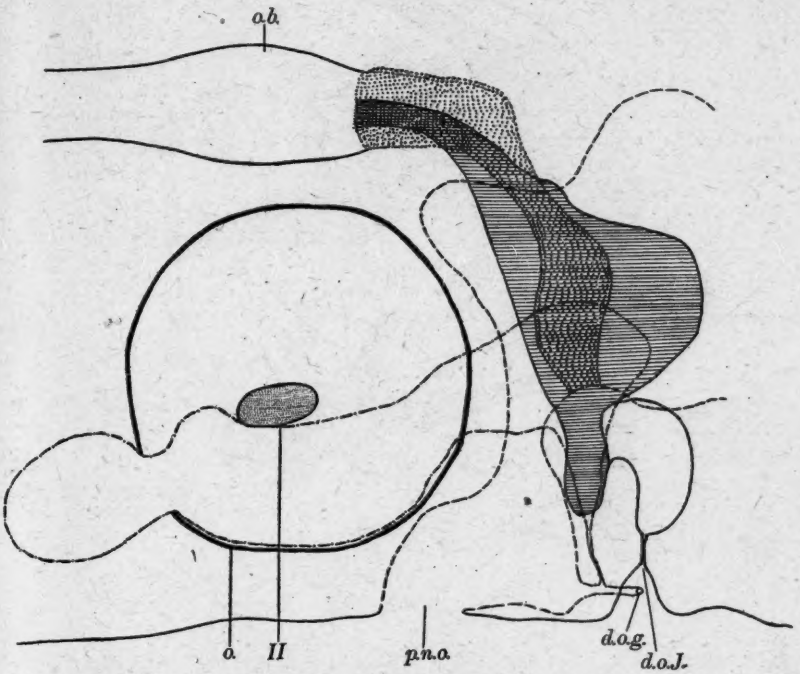


Fig. 3. Graphic reconstruction of right side of the orbito-nasal region. *d.o.g.* duct of orbital gland. *d.o.J.* duct of Jacobson's organ. *o.* boundary of orbit. *o.b.* olfactory bulb. *p.n.o.* posterior nasal opening. *II.* optic nerve. Horizontal lines: fibres derived from vomeronasal epithelium. Dotted lines: fibres derived from olfactory epithelium. Interrupted lines: outline of olfactory epithelium. Interrupted lines and dots: outline of orbital gland and its duct.

In *Chrysemys* the fibres arise from the vomeronasal epithelium (whose exact extent is in doubt), and course upwards and backwards to the roof of the nasal fossa, where they are joined by the olfactory nerves and the vomeronasal nerve of the opposite side. In the posterior nasal canal the vomeronasal and olfactory nerves are contiguous though their fibres do not intermingle. In the cranium the vomeronasal fibres pass dorsal to the olfactory nerves and reach

the vomeronasal area at the dorsal and medial surface of the olfactory bulb, which is homologous with the accessory olfactory bulb of mammals. The vomeronasal fibres are the only fibres which this receives.

McCotter (1912) also made gross dissections of this nerve in an adult snake (species unnamed) and a lizard (*Heloderma suspectum*), and found that in both these forms the nerve arose as two large bundles which passed backwards on the medial surface of the olfactory bulb and finally terminated in it.

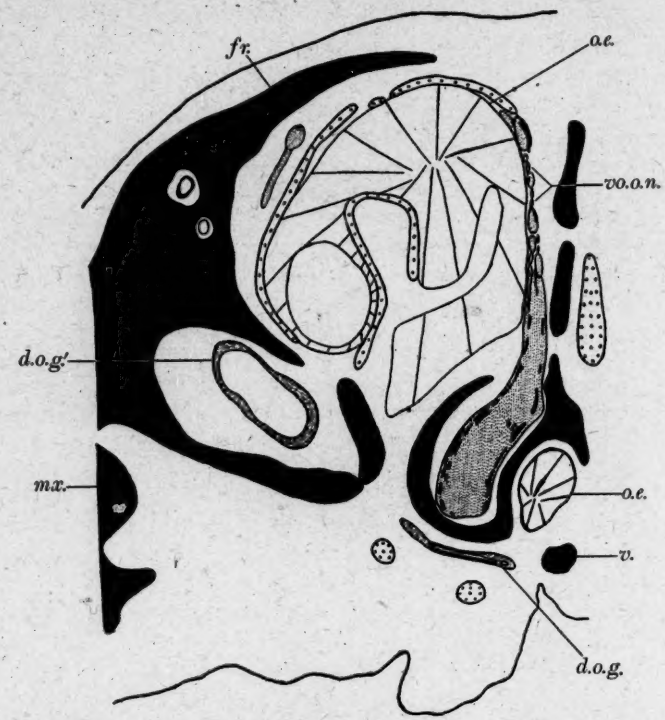


Fig. 4. Section showing issuing fibres of vomeronasal nerve. *c.* choroid. *d.o.g.* duct of orbital gland. *d.o.g.'* duct of orbital gland (upper part). *fr.* frontal bone. *o.* wall of orbit. *o.b.* olfactory bulb. *o.e.* olfactory epithelium. *o.g.* great orbital gland. *o.n.* olfactory nerve fibres. *mx.* maxilla. *p.* pigment. *p.f.* prefrontal bone. *tr.* trabecula. *v.* vomer. *vo.o.n.* combined vomeronasal and olfactory nerve fibres. *vo.n.* vomeronasal nerve fibres.

Wilde (1938) found that bilateral section of this nerve in *Thamnophis*, when the olfactory nerves were left intact, abolished the strike response in feeding.

Crosby & Humphrey (1938, 1939) made comparative studies of the accessory olfactory formation in several reptiles and other vertebrates. Their description of the relations of the vomeronasal nerve and accessory bulb in the crotaline *Agkistrodon* and the colubrines *Thamnophis* and *Natrix* agree closely with the conditions found in *Vipera*. They found that the size of the accessory

olfactory formation varied greatly in different groups of lizards, being large in *Varanus* and *Heloderma*, and very small in the Iguanids *Anolis* and *Phrynosoma*, where the organ of Jacobson is vestigial.

The connexions of the vomeronasal nerve were found to be similar in representatives of the marsupials, rodents, artiodactyls and carnivores by McCotter (1912). Humphrey (1940) has demonstrated the presence, differen-

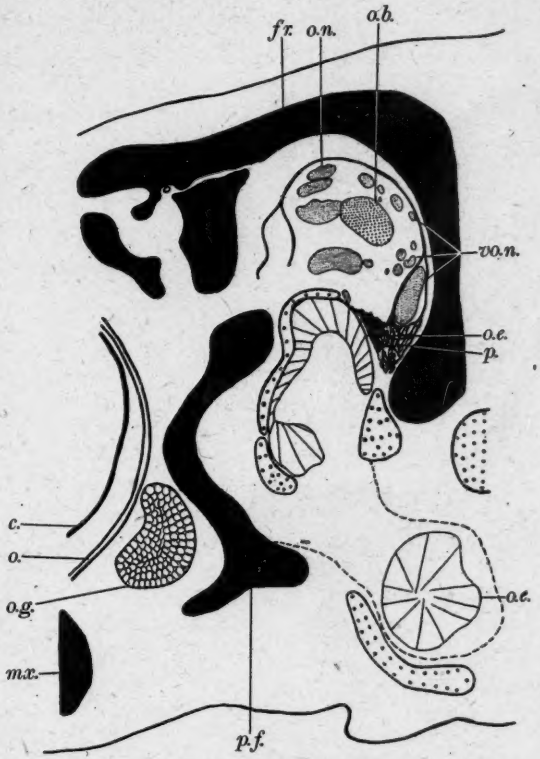


Fig. 5. Section through anterior boundary of orbit showing distribution of olfactory and vomeronasal fibres. Lettering as in Fig. 4.

tiation, and ultimate disappearance of the accessory olfactory formation and vomeronasal apparatus in a series of human embryos.

In *Vipera* the fibres arising from the neuroepithelial cells of Jacobson's organ are considerably more numerous than those arising from the olfactory epithelium proper, corresponding with the observations of Crosby & Humphrey (1938, 1939) upon *Agkistrodon*, where the accessory olfactory formation is larger than the true olfactory area. The separate bundles of the compound nerve were traced out, and as far as possible assigned to their respective

components, and a graphic reconstruction was made upon which Fig. 3 is based.

The vomeronasal nerve leaves the organ posteriorly as one tract (cf. McCotter, 1912), passes upwards through the roof of the capsule which is incomplete behind, and then runs upwards between the nasal capsule and nasal septum, where it is joined by the olfactory nerve whose fibre bundles are mingled with it (Fig. 4). It will be seen from Fig. 3 that the vomeronasal component extends forwards anteriorly to the level of the front of Jacobson's organ, and also extends farther posteriorly than does the olfactory nerve.

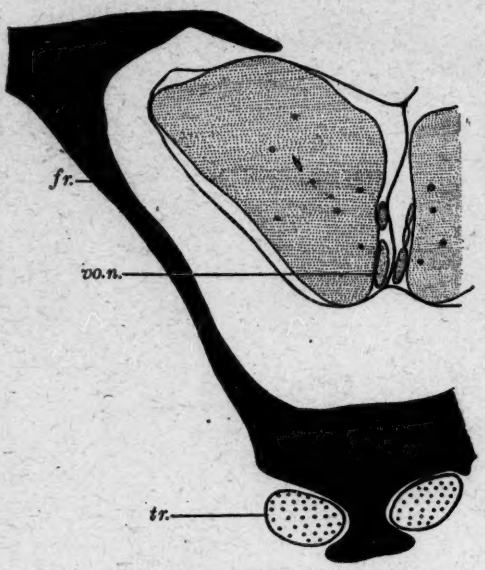


Fig. 6. Section through front of olfactory bulb. Lettering as in Fig. 4.

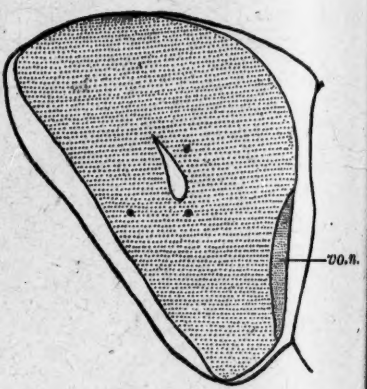


Fig. 7. The same as Fig. 6, a few sections further back. Lettering as in Fig. 4.

Dorsal to the nasal capsule the fibres separate, the vomeronasal fibres passing medially to the olfactory tract, which in Fig. 5 is just joining the olfactory bulb. Finally, the vomeronasal fibres pass along the medial side of the olfactory bulb to reach the accessory olfactory formation (Figs. 6, 7).

In its passage up the sides of the nasal capsule, the compound nerve is remarkable for the masses of pigment which lie among its fibres, and which also occur among the epithelial cells of Jacobson's organ itself.

The nervus terminalis (nerve of Locy)

The nervus terminalis was first described by Pinkus (1895) in the dipnoan *Protopterus*, and has since been identified in selachian, teleostean, holostean and dipnoan fishes. It is generally thought to be homologous with the apical

nerves of Van Wijhe in *Amphioxus*. A review of the literature up to 1936 in fishes is given by Kappers *et al.* (1936).

The relations of this nerve appear to be similar in all types observed. Johnston (1913) states that in fishes and Amphibia it is a ganglionated nerve whose root enters the forebrain caudal to the olfactory bulb beside the lamina terminalis, and is distributed to the nasal septum and vomeronasal epithelium when present. It occurs, however, in fishes where Jacobson's organ is absent and in certain reptiles and mammals, and in birds, where it is vestigial. In the posterior nasal canal it lies in close association with the olfactory tract, or with the vomeronasal nerve when this is present. Fibres have been traced to the nucleus preopticus, hypothalamus and nucleus interpeduncularis in fishes (McKibben, 1911) and in Amphibia (Herrick, 1933).

Ganglionic masses have been identified along its course in many forms, and, indeed, appear to constitute the main criteria for its identity. These ganglion cells have been shown to arise from the ventral region of the olfactory placode in the holostean *Amia* and other fishes by Brookover (1910) and Bonin (1939), rather than from the neural crest, as was held for *Squalus acanthias* by Locy (1899).

A ganglionated nervus terminalis has also been found in the larvae and adults of anuran and urodele amphibians, in the chick embryo, and in the embryos and adults of most orders of mammals, including man. The monotremes, which might yield interesting results, do not appear to have been investigated.

In reptiles, only the embryonic stages of Chelonia have been studied, and the nerve has been described in 10 and 20 mm. embryos of *Emys lutaria* by Johnston (1913), and in embryos up to 11 mm. of *Chrysemys marginata* by Larsell (1918*a*). In these turtles the nerve arises from a plexus on the nasal septum to which the trigeminal nerve also contributes, as in mammals, and passes along the vomeronasal nerve, becoming mingled with it and finally disappearing in the medial wall of the hemisphere behind the olfactory bulb. All along the course of the vomeronasal nerve are scattered at intervals groups of bipolar ganglion cells which were attributed to the nervus terminalis.

There is considerable doubt as to the exact nature of this nerve and the ganglion cells which occur in association with it. Johnston (1913, 1914) regards the nerve as probably containing afferent fibres in all types, and also probably efferent fibres to the nasal mucosa, but generally representing one component of a segmental nerve formed by it and the vomeronasal nerve, 'resembling the taste and general cutaneous components of VII and IX in some fishes'. Larsell (1918*b*) considered that it contained both general cutaneous and visceral sensory fibres from the septum, and also efferent fibres to the blood vessels, and comments on the resemblance of the nervus terminalis ganglia to sympathetic ganglion cells.

Huber & Guild (1913) also regarded these ganglia in rabbit embryos as sympathetic in nature, and probably containing both pre- and post-ganglionic

fibres to the septal mucosa and adjacent blood vessels. According to Brookover (1914) sympathetic connexions with the sphenopalatine ganglion and nerve occur in the full-time human foetus.

In *Vipera* a diligent search was made for cell bodies along the course of the vomeronasal and olfactory nerves, but none were found, and it was therefore concluded that the nerve was absent altogether, or had been incorporated within the compound vomeronasal and olfactory nerve and had lost its identity.

It seems possible that this absence of the nervus terminalis, in the adult viper, is associated with the absence of intrinsic glands and of a contractile cushion in the organ of Jacobson, the latter being supplied instead by the great orbital gland, which is innervated by branches of V.

SUMMARY

1. The structure and relations of Jacobson's organ and its duct in *Vipera berus* are described.

2. The duct of the great orbital gland was found to communicate with the duct of Jacobson's organ, and also with the nasal cavity.

3. The peripheral course and relations of the vomeronasal nerve are described.

4. No evidence was found for the existence of the nervus terminalis as a separate entity in the adult viper.

I am very grateful to Prof. J. D. Boyd for much help and advice, for access to the material, and also for providing the facilities without which these observations could not have been made.

REFERENCES

Bonin, W. (1939). *Arch. Biol., Paris*, **50**, 137.
Brock, G. T. (1929). *Quart. J. Micr. Sci.* **73**, 289.
Broman, I. (1920). *Anat. Hefte*, **58**, 168.
Brookover, C. (1910). *J. comp. Neurol.* **20**, 49.
—— (1914). *J. comp. Neurol.* **24**, 131.
Crosby, E. C. & Humphrey, T. (1938). *Mich. Acad. Sci. Arts & Let.* **24**, 104.
—— —— (1939). *J. comp. Neurol.* **71**, 121.
de Beer, G. R. (1937). *The Development of the Vertebrate Skull.* Oxford.
Francis, E. T. B. (1934). *The Anatomy of the Salamander.* Oxford.
Herrick, C. J. (1933). *J. comp. Neurol.* **58**, 1.
Huber, G. C. & Guild, S. R. (1913). *Anat. Rec.* **7**, 253.
Humphrey, T. (1940). *J. comp. Neurol.* **73**, 431.
Johnston, J. B. (1913). *J. comp. Neurol.* **23**, 97.
—— (1914). *Anat. Rec.* **8**, 185.
Kahmann, H. (1932). *Zool. Jb. Abt. Physiol.* **51**, 173.
Kappers, C. U. A., Huber, G. C. & Crosby, E. C. (1936). *The Comparative Anatomy of the Nervous System of Vertebrates Including Man.* New York: Macmillan.
Larsell, O. (1918*a*). *J. comp. Neurol.* **30**, 3.
—— (1918*b*). *J. comp. Neurol.* **30**, 423.
Looy, W. A. (1899). *Anat. Anz.* **16**, 273.

McCotter, R. E. (1912). *Anat. Rec.* **6**, 299.
—— (1917). *Anat. Rec.* **13**, 51.
McKibben, P. S. (1911). *J. comp. Neurol.* **21**, 261.
Noble, G. K. (1931). *The Biology of the Amphibia.* New York: McGraw-Hill Book Co.
—— (1937). *Bull. Amer. Nat. Hist. Mus.* **73**, 673.
Noble, G. K. & Clausen, H. J. (1936). *Ecol. Monogr.* **6**, 269.
Noble, G. K. & Kumpf, K. F. (1936). *J. Genet. Psychol.* **48**, 371.
Peyer, B. (1912). *Gegenbaurs Jb.* **44**.
Pinkus, F. (1895). *Morph. Arb.* **4**, 275.
Schwarz-Karsten, H. (1937). *Gegenbaurs Jb.* **80**.
Smith, M. A. & Bellairs, A. d'A. (1941). (Unpublished observations.)
Wilde, W. S. (1938). *J. exp. Zool.* **77**, 445.

THE EXISTENCE OF MICROGLIA IN THE NEUROHYPOPHYSIS

By E. VAZQUEZ-LOPEZ

From the Laboratories of the Imperial Cancer Research Fund, Mill Hill, London

The pars nervosa of the pituitary body is the only part of the central nervous system in which microglial cells have not hitherto been demonstrated. Search of the literature reveals only records of unsuccessful attempts. Bucy (1930), for example, found no microglia in the pars nervosa although in his studies of this portion of the ox pituitary he used the modification of Hortega's method which Penfield had specially devised for the impregnation of microglial and oligodendroglial cells. More recently Weaver and Bucy (1940) have actually used this supposed absence of microglia from the pars nervosa as a practical criterion in distinguishing tissue of this region from that of the adjoining tuber cinereum. Nevertheless, not only do microglial cells actually occur in the pars nervosa but they are present there in very large numbers. In the present paper the demonstration of these cells will be described and their peculiarities discussed.

That the microglia of the neurohypophysis should have remained undiscovered for so long a time is due to a combination of circumstances. Demonstration of microglial cells is always difficult no matter where they are found, but neither technical difficulties nor an unfortunate choice of animal species to be studied offer a complete explanation. A more important fact is that study of the elements of the pars nervosa has been comparatively neglected. Attention has centred on the pituitary body as an essentially glandular organ to the almost total exclusion of interest in other possible functions. In addition, there has been an unwillingness to visualize the pars nervosa as built up of typical nervous elements; witness the tendency to regard the neuroglia, already known as a structural element of this same region, as being here essentially different in character from the neuroglia found in other parts of the central nervous system. Fifty years ago Cajal showed that the neurohypophysis contains nerve fibres in extraordinary abundance but, in spite of the publication of numerous confirmatory reports, scepticism of the existence of these fibres is still occasionally expressed.

The present study has been directed mainly to conditions found in the horse. Pituitary bodies taken from newly killed animals were fixed in formol bromide for 2 days and longer. The method of staining was Hortega's original silver carbonate method in its several variations. Penfield's modification with hydrobromic acid has proved of special value for material fixed longer than 1 week.

These silver-impregnated preparations of the neurohypophysis of the horse regularly show the presence of a very large number of scattered cells which are all of like kind and which differ from the gliocytes in being of smaller size, in having smaller and denser nuclei, and in possessing scant elongated cytoplasm which extends in a variable number of slender processes of special type. Sometimes, especially when fixation has been limited to 2–3 days, these particular cells are impregnated with great selectivity and then they are seen standing out sharply against an almost unstained background which is formed by the rest of the tissue elements (Pl. 1, fig. 1). Reasons will be given for regarding the cells thus revealed as the microglia of the region and, to assist clarity of description and discussion, the cells will be called microglial cells from now on.

These microglial cells are distributed throughout the parenchyma of the pars nervosa in much the same numbers and in much the same manner as microglial cells in the central nervous system generally. Somewhat greater concentrations occur around blood vessels of medium calibre and in these situations the cytoplasmic prolongations tend to be more numerous. Similarly, in those parts of the parenchyma which are immediately subjacent to the pars intermedia the microglial cells are both more numerous and more richly furnished with cytoplasmic processes, but here, in addition, there is a distinct tendency for the processes to stretch out towards the neighbouring epithelial cells and thus to become orientated in a direction more or less perpendicular to the epithelial layer. In some parts of the parenchyma elements with fewer and shorter processes predominate; such is the case in the parts near the stalk, where the presence of bundles of nerve fibres imposes a somewhat longitudinal orientation on the microglial cells.

When the microglial elements of the pars nervosa are compared in greater detail with the microglial elements ordinarily found in typical parts of the central nervous system—the cerebral cortex for example—very considerable and characteristic morphological differences are found. Some few of the cells have very long slender processes and are closely similar to cortical microglial cells, but taken as a whole the microglial cells of the pars nervosa, though they vary much amongst themselves, are characterized by a relative abundance of cytoplasm, the cell processes being less numerous and thicker than in the corresponding cells of the cortex. The thicker, shorter, and fewer processes of the microglial cells of the pars nervosa are particularly characteristic. The processes themselves branch more rarely than those of more typical microglial cells and lateral spines are scarce or even almost absent. In the more extreme forms, which are far from rare, the elongated cell body stretching out beyond either end of the nucleus gives a rod-like form to the cell which now corresponds exactly to the 'rod cell' found in the various parts of the brain in pathological conditions. Indeed, if resemblances are being sought, the more profitable comparison is not with the microglial cells ordinarily found in typical parts of the central nervous system (that is with cells in the normal resting condition), but is rather with microglial cells in a more active state. The special features

which characterize the microglial cells of the normal pars nervosa are in fact exactly those which in other parts of the brain characterize microglial cells which are in a state of migratory activity and which have passed from the resting stage into the early phases of that sequence of pathological changes which will ultimately end in their becoming transformed into 'fat granular cells'. Up to and including the rod stage, Hortega's (1919, 1932) description of the initial changes which microglia undergoes in pathological cases involves a description of cellular forms which correspond exactly to the forms of microglial cells found dominating the picture in the normal neurohypophysis. Discussion of conditions known to affect the form assumed by microglial cells is clearly apposite since it may suggest the reason for the characteristic appearances presented by the normal microglia of the pars nervosa.

It is well known that cell forms differing from those of the normal resting microglia of the brain in general are not necessarily an expression of pathological conditions. Most often such changes are an expression of functional activity—cell motility or cell migration—occurring under what may be termed ordinary physiological conditions. Thus in the embryo the microglioblasts migrating from the vascular adventitia of the brain and from the meningeal folds—their chief source—appear first in the form of amoeboid or pseudopodic cells and then, after passing through a series of intermediate forms, finally reach the resting stage. In most species the earlier, simpler forms occur almost solely in the embryo and the very young (if the animal remains in normal health), but in some species, the fowl for instance, microglial migration from the perivascular adventitia seems to continue throughout life and migrating amoeboid cells are present in the adult in considerable numbers (Vazquez-Lopez, 1936).

It is recognized, too, that the physical consistency of the immediate cell environment plays its part in determining what form is taken by microglial cells. Costero (1931) studied tissue cultures of microglial cells and noticed a pronounced tendency to the formation of amoeboid and migratory forms exactly similar in morphology to those commonly known by these names in brain histology, and similar to the cells already described by these terms in the present paper. Costero concluded that explanted microglial cells assumed amoeboid and migratory forms because the medium in which they were growing was such that cell motility and migration were comparatively unrestrained.

The question arises whether either of these known factors—a chemotactic stimulation to mobilization, or a decrease in the physical resistance offered by the cell environment—can explain the morphological peculiarities exhibited by the microglia of the pars nervosa. A satisfactory explanation would give important support to our assumption that these cells are indeed microglial and are homologous with the microglial cells of the rest of the central nervous system.

Hoenig (1922) pointed out that the substance of the pars nervosa, unlike tissue taken from other parts of the brain, has a marked capacity for the

imbibition of water. Cushing (1933) drew attention to the peculiar 'juicy' character of the neurohypophysis and the related centres in the hypothalamus, and he attributed this peculiarity to impregnation with holocrin secretion derived from the epithelium of the pars intermedia. The presence of 'colloid' in the interstices of the pars nervosa was reported by Herring (1908) whose findings have been confirmed by Costa (1923), Collin (1924), Roussy & Mosinger (1933), Popjak (1940) and others. Gersh & Tarr (1935) deny the existence of such a secretion but claim to have shown that the neurohypophysis is infiltrated with a substance which is peculiar to this region, having the characteristics of an albumen, and which, under certain conditions, can be stained.

There is good evidence then that the substance of the pars nervosa differs in consistency from that of more typical parts of the central nervous system in being softer, more yielding in character, and in containing interstitial substance of more fluid nature. These, as we have seen, are just the conditions under which acknowledged Hortega microglial cells might be expected to approach the amoeboid form, a fact which offers a plausible explanation of the observed morphological peculiarities of the microglial cells of the pars nervosa.

But the true nature of the microglial cells of the pars nervosa is most clearly established by the prevalence of intermediate types in neighbouring parts of the hypophysis and its associated hypothalamic centres. Indeed, as one passes up through the stalk and on into the brain a gradual transition in type is seen, the form characteristic of the pars nervosa presently giving place to the resting stage characteristic of the central nervous system generally.

In the hypophysial stalk and in the more distal portion of the median eminence of the tuber cinereum the microglia does not differ materially from that of the pars nervosa (Pl. 1, fig. 2). In the more proximal part of the median eminence fewer microglial cells are demonstrable, but this may be due to the technical difficulties of impregnation since here the cells lie in a dense mass of neuroglial fibres. Most of these neuroglial fibres originate in ependymal cells lining the lower part of the third ventricle; they form a palisade perpendicular to the outer surface of the nervous tissue and end in enlarged feet which are in contact with the pars tuberalis. The shape of the microglial cells is here largely conditioned by the shape of the spaces between the neuroglial fibres. In the tuber cinereum, and especially along the ventricular walls, the microglial forms are clearly of intermediate type. Compared with the corresponding cells of the pars nervosa the cells in this region have processes which are longer, thinner, and more branched, though they still show a sharp double contour and lateral spines are still almost absent. The cell body is still somewhat thick and gross and still retains some irregularity of contour and an appreciable amount of granular cytoplasm which contrasts with the fineness, tenuity and delicacy of the cell bodies of cortical microglial cells; in the latter it is usually difficult to detect the extremely thin layer of perinuclear protoplasm from which the filiform and profusely branched cell-processes originate.

The neuroglia of the somewhat scattered areas that contain the supra-optic nucleus (the source of many of the fibres which innervate the posterior lobe) is characteristic of these parts and deserves more attention than it has hitherto received. It represents a transitional form which is a still closer approach to the resting types found in typical parts of the central nervous system. Pl. 2, fig. 3 illustrates conditions which obtain in the ventromedial portion of the supra-optic nucleus. The microglial cells are distributed fairly evenly throughout the extremely rich capillary network of this area. The cells still show a larger amount of cytoplasm and have processes which tend to be stouter and less branched than those found in normal resting microglia. The nodular swellings in the cell-processes constitute an outstanding feature in this area. The gradual transition of microglial types found in passing from the infundibular process through the pituitary stalk, median eminence, and tuber cinereum to the supra-optic nucleus is illustrated in Pl. 3.

Perhaps it is significant that all these regions characterized by this exceptional type of neuroglia—supra-optic nucleus, tuber cinereum, pituitary stalk and pars nervosa—have exceptional anatomical and physical characters in common. Vascularity is high and with proximity to the ependyma there is an abundance of neuroglial cells and fibres. But probably functional unity is the most important correlating factor associated with microglial type. The regions are those of the origin, course, and termination of the fibres which innervate the posterior lobe. They are remarkable, too, in that here the tissues are impregnated with substances, probably secretory in nature, which, if present at all, do not occur elsewhere in anything like the same concentration. It is also possible that morphological peculiarities of the microglial cells of these regions are associated with constant functional activity. The inclusions so common in the cell bodies, and in even the most delicate of their processes, give granularity to the protoplasm and irregularity of contour to the processes, but deformities caused by these inclusions cannot of themselves account for the whole of the morphological peculiarities. However, the inclusions do indicate great phagocytic and transportational activities, which are known to be among the functions characteristic of microglia in general and which are found most strongly exhibited in cells which, like the microglia of the neurohypophysis, have the morphology of the wandering phase.

Mention has already been made of the increased concentration of microglial cells in the pars nervosa along the line of its junction with the pars intermedia, and of the tendency for the cell processes to become orientated in a direction at right angles to that boundary. Successfully stained preparations also show that, creeping between the epithelial cells of the neighbouring part of the pars intermedia, there are elongated cells with almost filiform cytoplasm. For reasons to be given immediately these cells are regarded as migrating cells, derived from microglial cells which have crossed the border from the pars nervosa. It seems unnecessary to assume that all these cells have passed through the substance of the connective tissue of the capsule itself, since the

extremely numerous interruptions for the passage of blood vessels present an easy route.

Cajal's sublimate-gold method is generally used to reveal neuroglial cells of the central nervous system. In most animals the method is highly specific for these cells, but in the horse it is specific only for neuroglia of the brain. In the neurohypophysis of the horse the neuroglial cells are almost unstained as a rule, whilst microglial cells are often sufficiently well stained to permit of ready identification. Cajal's sublimate-gold method is therefore particularly useful in the study of these wandering cells of the pars intermedia of the horse; they are often particularly well shown, standing out vividly amongst almost colourless epithelial cells. The staining of these wandering cells is generally too dense to permit details of cell structure to be made out clearly; nevertheless such preparations give information supplementary to that obtained by silver impregnation. Accumulations of wandering cells are found at certain points, especially around the colloid cysts of small and medium size which are so plentiful in those parts of the pars intermedia which border on the pars nervosa. In such accumulations the length and tenuity of the cell processes and the intensity of the staining make it impossible to recognize the limits of individual cells (Pl. 2, fig. 4). Study of wandering cells is easier where they are less numerous, for in these areas they are found separated and isolated by the epithelial cells. Their filiform appearance is characteristic. They look as if compressed between the epithelial cells, and they have enlargements at the extremities when the existence of intercellular crevices permits.

The literature contains many references to cells which seem identical with these wandering cells though their true nature has not previously been recognized. Most probably these are the cells which Cajal, Gemelli and others, studying preparations made by Golgi's method, represent as very intensely impregnated isolated cells lying among colourless or faintly stained epithelial cords. Retzius (1894) described in some detail a branched type of 'Epithelzellen ähnelnde Zellen' in the pars intermedia and the two cells on the extreme left of fig. 4 in his monograph have the appearance of wandering elements. His description suggests that he was not convinced of the epithelial nature of these cells. Herring (1908), studying the cat, described cells which are situated between the epithelial cells and which he regarded as supporting elements. The characters of these cells are similar to the wandering cells found in the same situation in the horse.

A number of features support the opinion that these peculiar cells in the epithelium of the pars intermedia are indeed wandering cells derived from the microglia of the pars nervosa. Their morphological characters, their occurrence in crevices between the epithelial cells, and the readiness with which they become impregnated with silver, are all outstanding qualities of migrating phagocytic cells of which microglial cells are a type. The unevenness in distribution also is suggestive of wandering character. These cells in the epithelium of the pars intermedia are distinctly more numerous, for instance, around the

colloid cysts, the site of origin perhaps of the stimuli exciting them to mobilization. The cells become progressively less numerous as distance from the pars nervosa boundary increases until, near the pars anterior (distalis), they have almost disappeared. The fact that these wandering cells of the pars intermedia are relatively crowded along one side of the boundary, while highly amoeboid forms of microglial cells are similarly crowded along the other side, completes a histological picture which carries conviction that these wandering cells of the pars intermedia are simply microglial cells which have migrated across the border. The stimulus to migration is unknown but the constancy and extent of the phenomenon suggest an importance in relation to the function of the organ. But whatever the explanation, the fact of migration across the boundary between pars nervosa and pars intermedia is additional evidence of the very close functional relationship between these two regions of the hypophysis.

Up to this point only conditions found in the horse have been described and discussed, for, though pituitary bodies of both rabbit and ox have also been studied with care, the preparations have always been less successful and therefore less informative. Lack of real success seems completely explicable on technical grounds, and the evidence so far obtained suggests that microglia is present in the pars nervosa of both ox and rabbit and that, in essentials, conditions in these animals are much the same as in the horse.

Because of its relatively loose texture and the simple arrangement of its tissue components the pituitary body of the horse must be regarded as a material of choice for histological studies. In both ox and rabbit the pars nervosa has a closer texture. In the rabbit the crowding together of tissue elements is due to an abundance of neuroglial cells and of bundles of nerve fibres lying in between the perivascular spaces which themselves contain much fibrous tissue. In the pars nervosa of the ox there is an extraordinary abundance of connective tissue throughout the organ and this leaves relatively little room for other tissue elements. In view of the greater concentration of tissue elements in these two species and of the extraordinarily complex intermingling of collagenous fibres, reticular fibres, nerve fibres and neuroglial cells with long cytoplasmic prolongations, it is perhaps to be expected that silver impregnation of microglial cells should prove exceptionally difficult. Methods for the demonstration of microglia are erratic under the best of conditions, but here in the ox and rabbit the microglial cells are deeply buried among other tissue elements, some of which have an avidity for the reagents used. To reveal the delicate microglial cells, even imperfectly, demands more careful and patient work than is usually found possible in the ordinary routine work of a laboratory, and to show them satisfactorily under these more difficult conditions may necessitate the development of staining methods more highly selective than those available at present. But sufficient has been achieved to show the presence of elements which probably represent the Hortega microglial cells. In the pars nervosa of the rabbit cells can occasionally be found sufficiently well impregnated and sufficiently complete to permit of definite identification. In the pars nervosa

of the ox the best that has been possible is the staining of small, irregular, elongated nuclei containing granular chromatin and highly suggestive of microglial cells. But, notwithstanding this scant success in the demonstration of microglia in the pars nervosa of other species, it is considered that the successful demonstration, described at length in the present paper, that microglial cells exist throughout the neurohypophysis of the horse, that they are present in numbers comparable with those of cells in typical parts of the brain, and that they form a microglial system which is continuous with that of the neighbouring portions of the central nervous system, constitutes sufficient ground for the opinion that in general the nervous portion of the hypophysis cannot be distinguished and delimited from other parts of the brain by absence of microglial cells.

SUMMARY

1. Microglia is shown to be present in the neurohypophysis of the horse. It is continuous with the microglia of the adjacent parts of the central nervous system. The microglial cells are distributed throughout the region and are present in much the same numbers as microglial cells in more typical parts of the brain.

2. In the neurohypophysis and in the hypothalamic centres with which it is both anatomically and functionally related, the microglial cells present characteristic morphological features. They correspond to the forms found elsewhere in the initial stages of microglial mobilization.

3. These characteristic morphological features of the microglial cells are probably associated with the peculiar physico-chemical constitution of the neurohypophysial substance and with an exceptional degree of cell activity.

4. Between the epithelial cells of the pars intermedia, especially near its junction with the pars nervosa, there are peculiar cells which are probably migrated microglial cells.

5. There is evidence that in other species, too, microglial cells are present in the pars nervosa, and the tentative general conclusion is drawn that with regard to the occurrence of Hortega microglial cells the pars nervosa of the pituitary body does not differ essentially from the rest of the central nervous system.

Thanks are offered to Dr W. E. Gye, F.R.S., Director of the Laboratories, for constant help and encouragement received throughout the course of this work; to Mr R. E. Glover, F.R.C.V.S., of the Farm Laboratories of the National Institute for Medical Research, for generous gifts of the fresh horse and ox pituitaries which have been used; to my colleague, Dr W. J. Purdy, for help in the preparation of the manuscript and to Mr A. V. Cobbett of the laboratory technical staff for the photomicrographs and drawings.

REFERENCES

Bucy, P. C. (1930). *J. comp. Neurol.* **50**, 505–20.
Collin, R. (1924). *C.R. Soc. Biol., Paris*, **91**, 1334–5.
Costa, A. C. de (1923). *C.R. Soc. Biol., Paris*, **88**, 833–5.
Costero, I. (1931). *Z. ges. Neurol. Psychiat.* **132**, 370–406.
Cushing, H. (1933). *Amer. J. Pathol.* **9**, 539–48.
Gersh, I. & Tarr, A. D. (1935). *Anat. Rec.* **63**, 231–8.
Herring, P. T. (1908). *Quart. J. exp. Physiol.* **1**, 121–60.
Hoenig, Ch. (1922). *Z. ges. Neurol. Psychiat.* **79**, 197–209.
Popjak, G. (1940). *J. Path. Bact.* **51**, 83–90.
Retzius, G. (1894). *Biol. Untersuch.*, Neue Folge, **6**, 21–4.
Rio Hortega, P. del (1919). *Bol. Soc. esp. Biol.* **9**, 69–120.
—— (1932). Penfield's *Cytology of the Nervous System*, **2**, 482–534. New York: Hoeber.
Roussy, G. & Mosinger, M. (1933). *C.R. Soc. Biol., Paris*, **112**, 1203–4.
Vazquez-Lopez, E. (1936). *Arch. Psychiat. Nervenkr.* **104**, 652–62.
Weaver, T. A. & Bucy, P. C. (1940). *Endocrinology*, **27**, 227–35.

EXPLANATION OF PLATES 1–3

Plate 1

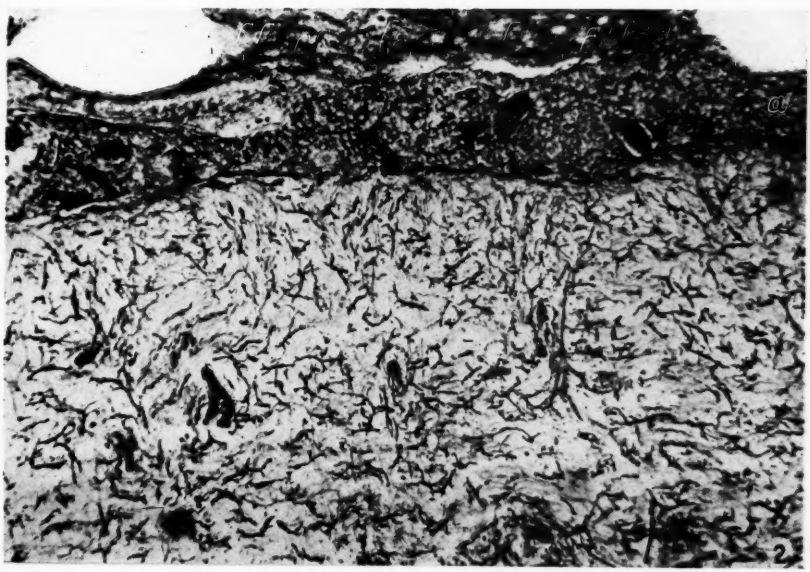
Fig. 1. Microglia in the nervous lobe of the hypophysis.
Fig. 2. Microglia in the upper part of the stalk. *a*, pars tuberalis.

Plate 2

Fig. 3. Microglia in the supra-optic nucleus.
Fig. 4. Wandering cells of the pars intermedia. *a*, pars nervosa. Cajal's gold sublimate method.

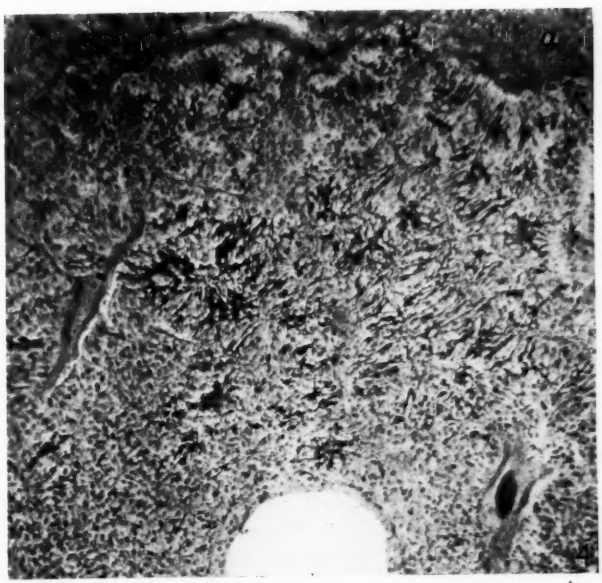
Plate 3

Showing the gradual transitions of microglial type. (A) pars nervosa, (B) tuber cinereum, (C) supra-optic nucleus.



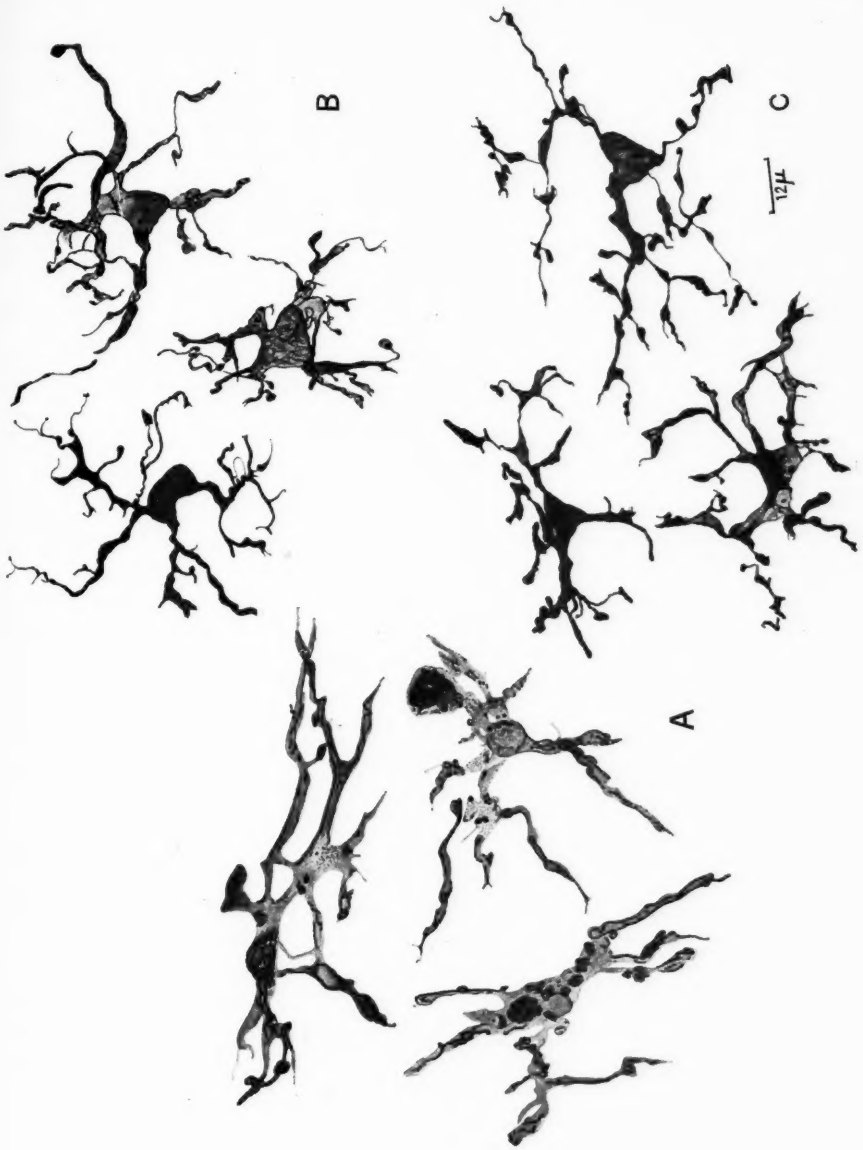
VAZQUEZ-LOPEZ—THE EXISTENCE OF MICROGLIA IN THE NEUROHYPOPHYSIS

UNIV. OF MICH.



VAZQUEZ-LOPEZ—THE EXISTENCE OF MICROGLIA IN THE NEUROHYPOPHYSIS

UNIV. OF MICH.



VAZQUEZ-LOPEZ—THE EXISTENCE OF MICROGLIA IN THE NEUROHYPOPHYSIS

UNIV. OF MICH.

A PRESOMITE HUMAN EMBRYO (SHAW): THE IMPLANTATION

By W. J. HAMILTON and R. J. GLADSTONE

Department of Anatomy, St Bartholomew's Hospital Medical College, London

INTRODUCTION

In a previous communication (Gladstone & Hamilton, 1941) we gave a short description of the chorionic vesicle, and a detailed account of the structure of the intrachorionic rudiment of the 'Shaw' embryo. The present communication provides a more detailed account of the chorionic villi and their method of attachment to the implantation cavity. An account is also given of the structure of the endometrium adjacent to the implantation cavity.

The chorionic vesicle has the following diameters:

Maximum external, including villi: 11 mm.
Vertical external: 4·04 mm.
Maximum internal: 8 mm.
Vertical internal: 3 mm.

The wall consists of an inner mesodermal layer (a description of this tissue is given in our previous paper), a cytotrophoblastic or Langhan's layer, and a syncytiotrophoblastic layer. Projecting from the surface of the vesicle throughout its extent are villi; these are more differentiated and longer at the circumference than at the poles of the chorionic vesicle. The villi may be divided into three parts, a proximal part which has a mesodermal core, a short intermediate part which is densely cellular, some of the cells of which are continuous with the cytotrophoblast, and a distal part composed of loose columns of cytotrophoblastic tissue. There is a layer of syncytiotrophoblast of varying thickness on the sides of the villi.

As there is some confusion in the literature on the nomenclature of the different parts of the trophoblastic tissue we give here an account of the terms which we use:

Plasmoditrophoblast we regard as the primary plasmodium seen at the early stages of implantation, e.g. Miller, T.B.1, Peters, and other early embryos; it corresponds to the implantation syncytium of Grosser's classification (1927) and to the plasmodium of Florian (1928). After implantation it undergoes retrogression and, in the present specimen, only remnants of it are present.

Syncytiotrophoblast has been divided into villous syncytiotrophoblast, which covers the villi, and peripheral, which is found on the trophoblastic shell and lining the implantation cavity; it corresponds to the resorptive syncytium of Grosser (1927) and Florian (1928).

Cytotrophoblast, following the description of Ramsey (1938), has been subdivided into central cytotrophoblast, or Langhan's layer, and peripheral, or cytotrophoblast of the cell columns and trophoblastic shell. This is regarded as a second generation of cytotrophoblast by Grosser (1927).

The *trophoblastic shell* is recognized as the layer of cytotrophoblastic tissue, of varying thickness, which lines the implantation cavity including the decidua capsularis.

Peripheral syncytium is regarded as the tissue which invades the maternal tissue and which is situated outside the trophoblastic shell. It corresponds to the syncytial sprouts of Hill (1932) and the proliferation plasmodium of Florian (1928). It is typically found outside the border, or penetration zone, or the foetal-maternal border, of Wislocki & Streeter (1938).

THE IMPLANTATION CAVITY

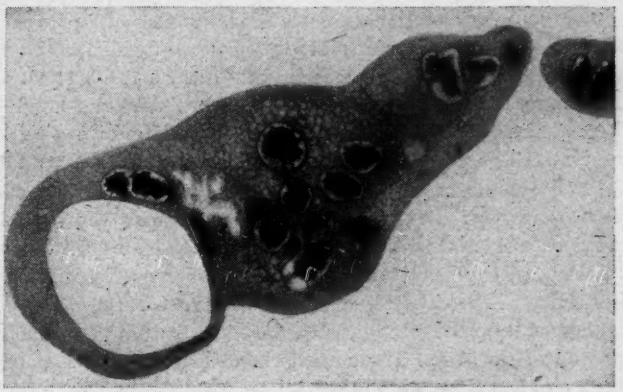
A general view of the specimen shows that the walls of the implantation cavity have been torn, more especially on the superficial aspect and at the sides (Pl. 1, fig. 1). At one side the decidua capsularis has been torn away from the tips of the chorionic villi and has become folded and broken. A large fragment includes nearly the whole of the 'operculum deciduae' and the zone of the decidua capsularis surrounding the infiltrated fibrinous plug which has sealed the aperture of entry. On the deep aspect or placental side of the chorionic vesicle the wall of the implantation cavity is formed by an incomplete layer of cytotrophoblastic cells. Syncytiotrophoblast covers the foetal aspect of the cytotrophoblastic lamina, and scattered plasmoditrophoblast masses are found over and amongst these cells. A large cleft, the maternal sinus, separates the cytotrophoblastic lamina from the maternal decidua basalis. The maternal sinus, which is lined with endothelium in some areas and in others by flattened trophoblastic cells, opens into intervillous spaces.

Since considerable variations exist in the structure of different parts of the wall of the implantation cavity, it is desirable to consider these parts under separate headings, namely: (1) A basal or placental area. (2) A marginal zone or circumferential part of the decidua capsularis, forming the lateral walls of the implantation cavity. (3) The central part of the decidua capsularis or superficial wall next the lumen of the uterus, comprising the 'operculum deciduae', the infiltrated fibrinous plug, and the circular zone of the decidua capsularis surrounding the 'aperture of entry'.

(1) *The basal or placental area.* A wide space, the basal sinus, separates the outer wall of the implantation cavity from an incomplete lamina which is formed by fused plates of cytotrophoblastic tissue uniting the tips of the villi. These plates arise from, and are continuous with, proliferating cells constituting the cell columns which grow outward from the tips of the villi. The cells at the base of the cell columns are continuous with the central cytotrophoblastic layer of the villi (Pl. 1, fig. 2). The central cytotrophoblastic cells lining the bases of the villi are cuboidal and are continuous with the similar cells in the chorionic wall. They have a large, round nucleus, with a distinct nucleolus, and a small amount of pale cytoplasm; mitoses are frequently found in these cells (Pl. 2, fig. 3). The cells of the peripheral cytotrophoblast which form the cell columns and the cytotrophoblastic lamina vary in size, those close to the tips of the villi being smaller than the more peripherally situated cells. The cell boundaries

are less clearly defined, the nuclei are paler and often irregular and are surrounded by a dark band of cytoplasm outside of which there is a clear space (Pl. 2, fig. 5). Mitoses are not frequently observed in these cells.

The central cells are covered by a complete layer of syncytiotrophoblast, patches of which are occasionally included in the cytotrophoblast. Remnants of degenerating maternal tissue are rarely to be found among the cytotrophoblastic cells and occasional leucocytes are also present. The syncytiotrophoblast of the villi and chorionic wall varies in thickness in different villi; in some areas the outer border contains small vacuoles giving it a foamy appearance; in other areas large vacuoles are found (Pl. 2, figs. 3, 4). The syncytiotrophoblastic layer stains more darkly than the cytotrophoblast cells. A typical 'brush border' was observed in many parts (Pl. 2, fig. 4); in others it is indistinct or absent.



Text-fig. 1. Remnants of the plasmoditrophoblast, or primary plasmodium of the implantation. Note large and small vacuoles and pycnotic nuclei in process of disintegration. ×*c.* 550.

The nuclei vary in size and are irregular in shape; where the layer is thin they are flattened. Only very rarely are mitotic figures seen. A distinct nucleolus is present. In some situations the syncytiotrophoblastic layer shows indefinite cell boundaries (Pl. 2, fig. 3). The isolated masses of plasmoditrophoblast stain a deep pink colour with eosin, often with a lavender tint. Many small vacuoles and well-formed nuclei are present in certain of these masses, while other pieces contain large vacuoles and degenerating nuclei (Text-fig. 1). Large, apparently anuclear, masses are also present. Occasionally engulfed maternal blood cells and other maternal tissue are found within the masses. No transitional stages between the syncytiotrophoblast and the plasmoditrophoblast can be demonstrated.

The greater part of the uterine wall of the sinus is lined by a single layer of epithelioid cells continuous with the endothelium lining maternal vessels which open into the sinus (Pl. 1, fig. 2). Areas, however, are present which

are covered by laminated masses of fibrin. In some places the epithelioid layer lies beneath a laminated fibrinous deposit, and in others the fibrinous deposit is infiltrated with nuclei indicating commencing organization of a clot which had been deposited during life. Where anchoring villi are seen directly attached to the decidua, near the circumference of the placental zone, there is no large continuous space or sinus, the intervals between the attachments of the villi being simply radiating clefts, lined by syncytiotrophoblast, which extend a short distance into the maternal tissue in the zone of penetration. It is probable, therefore, that, in the living state, the space existed as a cleft which was capable of opening up into a sinus containing a variable amount of maternal blood which was in direct contact with the syncytiotrophoblast lining the uterine wall of the sinus as well as with that covering the villi and the outer wall of the chorionic vesicle.

The outer boundary of the sinus shows a number of rounded swellings separated by grooves or depressions into the bottom of which capillary vessels, and spaces lined by endothelium, are seen to open (Pl. 1, fig. 1; Pl. 4, fig. 10). The endothelium lining these vessels is continuous with a single layer of syncytiotrophoblast which in most places covers the summits of the swellings. The character of this epithelial lining is, in general, that of an attenuated layer, the nuclei and cytoplasm both being stained a deep purple colour and with no visible cell outlines; the nuclei of the tissue covering the summits of the swellings are as a rule larger and more rounded than the nuclei of the endothelium lining the capillary vessels and vascular spaces. Further, there is direct continuity of the thick layer of syncytiotrophoblast, which covers the sides of adherent 'anchoring' villi, with the thin stratum of epithelium lining the outer wall of the sinus. These anchoring villi are most numerous at the circumference of the sinus where the villi are firmly adherent to the decidua, whereas, in the central region, they are united together at their surfaces by expansions from the cell columns to form cytotrophoblastic laminae.

In some places the syncytiotrophoblast lining the uterine wall of the sinus is seen to be continuous with the epithelium of distended and degenerating uterine glands which have been opened up by the formation of the implantation cavity (Pl. 4, fig. 8). Many of these glands are greatly elongated and displaced, so that their long axes lie parallel to the outer wall of the sinus. Since their much enlarged lumina often contain blood as well as secretion, it is probable that when, in the earlier stages of the embedding process, the interglandular tissue in the 'necrotic zone' breaks down, the opening up of their lumina contributes to the formation of the cavity of the sinus. In many places groups of chorionic villi are attached to a thin plate of cytotrophoblastic tissue, covered by syncytiotrophoblast, which intervenes between the cavity of the sinus externally and the intervillous space internally, that is, on the side turned towards the wall of the chorionic vesicle. This clearly indicates, in our opinion, that a cleft has been formed by the breaking down of the spongy tissue composing the 'necrotic zone' described by Bryce & Teacher (1908) in the earlier

stage of development which follows the initial entry of the blastocyst. At the stage reached by the specimen we are describing, the necrotic tissue which formed the solid part of the sponge-work has broken down and has been supplanted by thin plates of cytotrophoblast covered by syncytiotrophoblast, which join the tips of small groups of chorionic villi and which contribute to the formation of the trophoblastic shell. Also at this stage both walls of the sinus are covered by syncytiotrophoblast which is thicker and more typical in structure where it covers the outer surface of the thin plates attached to the tips of the villi than externally where it is attenuated and in some places defective.

In the region of the sinus, and in relation with the outer or parietal wall of the implantation cavity in the future placental area, we can distinguish three surfaces which are disposed in concentric planes relative to the outer wall of the chorionic vesicle. These surfaces are: (1) The outer wall of the intervillous space. The thin plates to which the tips of groups of anchoring villi are attached are, in the vicinity of the villi, lined by syncytiotrophoblast. (2) The outer surfaces of the thin plates of cytotrophoblast are also partly covered by syncytiotrophoblast, more particularly where this has apparently grown round the edges of the gaps between the plates; elsewhere it is bare or covered by a single layer of endothelium-like cells, suggesting the formation of the sinus by the opening up of vascular spaces as well as by the inclusion of dilated uterine glands and breaking down of necrotic tissue. Bare areas are also present which are sometimes covered by blood clot or a fibrinous coagulum tinged with eosin and containing disintegrated red blood corpuscles and leucocytes. The third surface is the outer wall of the sinus. The syncytiotrophoblast lining the latter also varies in different places. In the clefts between the rounded swellings, where capillary vessels open into the sinus, the latter is lined by endothelium; where uterine glands have been opened up the epithelium lining the sinus is continuous with, and resembles, the glandular epithelium. In other places there are definite patches of plasmoditrophoblast giving origin to plasmodial strands which radiate outwards into the uterine tissue beyond the sinus where it may be seen opening up distended and degenerating glands, and extending into congested capillary vessels and vascular spaces.

(2) *The marginal zone or circumferential part of the decidua capsularis forming the lateral walls of the implantation cavity.* The marginal or circumferential part of the decidua covering the sides of the chorionic vesicle differs from the decidua basalis in being more dense and uniform in general appearance. The uterine epithelium on the surface is a simple layer of cubical and flattened cells beneath which are a considerable number of lymphocytes. The stroma differs from that of the decidua basalis in the sparseness of the glands, only remnants of which are present. The epithelium of the glands shows varying stages of disintegration and in some cases has completely disappeared. In other places the walls consist merely of flattened cells of mesothelial type. On reaching the angle between the marginal and the parietal deciduae the

lateral blood sinus divides into two branches of which one extends into the adjacent part of the decidua parietalis and the other is prolonged into the decidua marginalis and, by further extension and subdivision, into the peripheral part of the decidua capsularis. The sinuses are lined by flattened endothelial cells and contain blood coagulum, palely stained with eosin, in which are embedded scattered leucocytes and degenerated erythrocytes. The stroma contains a large number of round, oval and branched decidual cells which extend throughout the whole thickness of the stroma and are characterized by their large size, the pink colour of their cytoplasm, and their large, round nuclei (Pl. 3, fig. 7). The nucleus is palely stained by haematoxylin, the nuclear membrane is delicate and there is a fine nuclear reticulum showing usually a nucleolus and slight nodal thickenings. Numerous lymphocytes and leucocytes are present in the stroma and are most numerous beneath the uterine epithelium and around the blood sinuses. The stroma nearer the chorionic vesicle is denser and more uniform in appearance than it is nearer the surface and beneath the uterine epithelium, where it appears to be oedematous. The decidua marginalis is limited on its inner or chorionic surface by an incomplete layer of cytotrophoblast and some plasmodial remains. The tips of the elongated circumferential villi have become detached in many places, the points of attachment being merely indicated by bare areas or a few cytotrophoblast cells adhering to the inner surface of the decidua (Pl. 3, fig. 6). Syncytial processes extending into the marginal decidua are frequent and are best seen near the junction of the marginal with the central part of the decidua capsularis (Pl. 3, fig. 7). These syncytial processes vary in shape and are darkly stained in contrast to the more lightly stained decidual cells.

(3) *The central part of the decidua capsularis, including the 'operculum' and the region surrounding the aperture of entry.* The epithelium on the surface of the decidua capsularis consists of a single layer of low cubical or polygonal cells which are continuous with similar cells of the marginal zone; the cell bodies are stained pink with eosin, the cytoplasm being coarsely granular and frequently showing vacuoles which are most frequent in the vicinity of the nucleus and near the free border of the cell. The outlines of the cells are ill-defined and there is no definite basement membrane. The nuclei are stained a deep purple and are round or oval in outline. Mitotic figures are rarely seen, but lymphocytes lying in pairs close together are common immediately beneath the epithelial cells or, in some places, between the cells.

In the compact tissue beneath the epithelium are numerous decidual cells; these appear either as isolated cells or in groups and columns. The existence of the decidual cells is in most places associated with the presence of numerous lymphocytes and leucocytes in the supporting connective tissue. The interglandular connective tissue appears to have broken down in places, and in others to have been replaced by decidual cells. The presence of decidual cells at this stage of development is not limited to the zone immediately surrounding the implantation cavity, these cells being found also in the decidua parietalis,

a small portion of which, in our specimen, is in continuity with the decidua capsularis.

On approaching the region of the closed aperture of entry the uterine epithelium tends to become flattened. Transitional stages occur between a single layer of evenly disposed cubical cells and one of irregularly shaped flattened cells which is spread out over the stratum compactum. The cells here show signs of degeneration and appear to be stretched over the underlying compact layer. In some places the epithelium is absent, leaving bare areas limited by a stratum of condensed connective tissue, or 'basement membrane'.

Beneath the surface layer of uterine epithelium apparently isolated cells or small multinucleated masses of syncytium are occasionally visible and also a considerable number of lymphocytes and polymorphonuclear leucocytes; the stroma contains numerous decidual cells (Pl. 3, fig. 7). The latter, however, diminish in number as the aperture of entry is approached. Further, in this region all traces of the uterine glands have disappeared and the stroma becomes denser and less vascular. It is infiltrated with extravasations of blood and fibrinoid material and contains a large number of lymphocytes. The deep surface of the decidua capsularis is covered by cytotrophoblastic cells which are several layers thick in places, while in other areas they only appear as scattered cells. They line part of the trophoblastic shell.

The 'operculum deciduae', or closing plate. This is a flattened disc-like structure the margins of which spread out from the site of the original aperture of entry over the uterine epithelium which covers the stratum compactum of the decidua capsularis. The exact size of the operculum is difficult to determine as, in the sections, the edge of the disc on one side is seen to be adherent to the surface of the decidua capsularis and to thin out gradually into an indeterminate border, whereas on the other side, where it is still separate from the decidua, the border is folded. Further, the lengths of the diameters at right angles to the planes of the sections cannot be estimated with certainty since the edge of the operculum extends on both sides beyond the area contained within the block. The number of serial sections (which were cut at a thickness of 10 μ) is 263. This gives a minimum diameter in a plane at right angles to that of the sections of 2·63 mm. The greatest horizontal diameter of the disc, obtained by measurements from sections on the slides, is approximately 3 mm. Thus, assuming that the disc was roughly circular in outline, and allowing 0·37 mm. for the missing edges of the disc which were not included in the block, the average length of the diameters passing through the centre of the disc would be approximately 3 mm., or about one-third of the maximum diameter of the chorionic vesicle.

The operculum is seen in all the sections to rest on the surface of an accidentally detached fragment of the decidua capsularis. One edge of this fragment appears to have been torn from the circumferential part of the decidua capsularis a short distance from its junction with the decidua parietalis; here the torn edges of the two parts of the decidua capsularis which are slightly

separated are seen to correspond. The other end of the fragment gradually tapers to a free border which is at a considerable distance from the free border of the torn decidua on the corresponding side (Pl. 1, fig. 1). The wide gap between these two edges is occupied by a part of the wall of the chorionic vesicle, and from the latter project the elongated branched villi which characterize the circumferential zone of the vesicle. The tips of these villi are joined by thin plates of syncytiotrophoblast forming the trophoblastic shell, which are similar to the plates which have been previously described in the region of the decidua basalis. The superficial surface of these plates, however, although covered in some places by a syncytial layer, is in the greater part of its extent bare; it appears to have been torn away from the superficial layer of a missing part of the decidua capsularis which would have covered the exposed part of the chorionic vesicle.

The operculum consists of a hyaline fibrinoid material stained a yellowish pink colour with eosin and containing irregularly disposed, deeply stained, pycnotic nuclei (Pl. 4, fig. 9). These are round, oval, or rod-shaped, and vary in size, some being comparable to the nucleus of a small lymphocyte, though a cytoplasmic zone around the nucleus is seldom recognizable. Other nuclei are flattened and are seen mostly on the free surface; these resemble the nuclei of a mesothelium, while others which are larger in size appear to be the nuclei of degenerating plasmoditrophoblast. No vessels are visible, although where the operculum has become adherent to the decidua, ingrowths are present on its deep surface which resemble angioblastic strands and indicate a commencing organization of the fibrinoid material. Degenerating red blood corpuscles are visible in some places, and in some places also the ground substance or matrix is laminated, suggesting that the structure has originated as a blood coagulum which has become infiltrated with leucocytes and is undergoing degeneration and absorption. It is probable that the adherent portions of the operculum would have been replaced by scar tissue, while the outlying and circumferential free parts which do not receive any blood supply would have disintegrated and been cast off into the lumen of the uterus.

COMMENTS AND COMPARISONS

It is not our intention to enter into a detailed discussion of different phases of implantation, but only to deal with the more recent relevant literature. A statement on the age of the embryo was given in our previous communication. The assumed fertilization age of the embryo, from the patient's history, was estimated to be between 20 and 23 days. The embryo was found, however, to be much younger than its supposed fertilization age and was estimated to be at about the 17th or 18th day of development, i.e. at approximately the same stage of development as the Wa 17 (Grosser, 1931) and younger than the Jones & Brewer (1935). The chorionic villi show a stage of development and differentiation comparable to embryos of the Group E of Bryce's classification (1924), and this group would also include the additional embryos, Hugo (Stieve, 1926),

Goodwin (Kindred, 1933), that of Jones & Brewer (1935), of estimated age of 18½ days, and HR 1 (Johnston, 1941). The villi are found attached to the entire circumference of the chorion. The longest villi are found in the region of the circumference of the chorionic vesicle and in this respect it resembles the Goodwin embryo (Kindred, 1933). Stieve (1926) found, in the Hugo embryo, that the longest and thinnest villi were in the basal region and the thickest in the region of the decidua capsularis. In the embryo described by Brewer (1937) the villi were most numerous in the basal region. Johnston (1941) found, in embryo HR 1, that they were longest in the basal region but less branched than in the lateral region.

Each villus may be differentiated into a basal part with a mesodermal core, a distal part composed of loosely arranged cell columns, and an intermediate part which has more regularly arranged cells and which stains more intensely than the distal part. In the mesoderm of the base of the villi developing blood vessels are found (Gladstone & Hamilton, 1941). No blood vessels were found in the mesodermal core of the Goodwin embryo but in it the chorionic vesicle is relatively small.

It is now generally agreed that the cell columns growing from the villi are derived from the cells of the cytotrophoblast which covers the mesoderm. Frequent mitoses are found in the cytotrophoblastic cells at the bases of the columns, similar to those reported by Jones & Brewer (1935). In the cell columns, on the other hand, mitotic figures are rarely seen. The absence of mitotic figures in the cell columns in HR 1 embryo is noted by Johnston (1941), who infers that the cells may divide by amitosis as suggested by Florian (1928). We are of the opinion that the cellular activity of the cytotrophoblastic cells at the base of the columns is sufficient to account for the growth of the cell columns. Jones & Brewer (1935) were able to demonstrate the presence of glycogen in these cells. They also point out that the cells become larger and frequently show degenerative changes near the periphery of the cell columns. They further demonstrated, by using silver and Mallory's connective tissue stain, that the cell columns are of foetal origin; no connective tissue fibres are found amongst the columns.

On the basal side of the chorionic vesicle of the Shaw embryo the cell columns unite to form a distinct cellular lamina; towards the circumference of the vesicle and decidua capsularis they are in continuity with the cytotrophoblastic tissue of the trophoblastic shell, as is the case in the embryos Hugo (Stieve, 1926), Bi 1 (Florian, 1928), Falkiner (1932), and Yale (Ramsey, 1938).

Florian (1928) differentiates between two kinds of plasmodium, resorptive and proliferative. The former is subdivided into that in the villi and cell columns (corresponding to our syncytiotrophoblast), and isolated protoplasmic masses, corresponding to the plasmoditrophoblast of our description. The proliferative type is found in the penetration zone with elongated nuclei which are frequently pycnotic.

The syncytiotrophoblast forms an almost complete layer of varying thickness which covers the chorionic vesicle, the villi, the cell columns and the lacunae of the trophoblastic shell, as is the case in the Hugo embryo (Stieve, 1926). The amount of the syncytiotrophoblast varies in different areas; it is least in the trophoblastic shell and greatest on the sides of the villi. The transition of the cytotrophoblast into syncytiotrophoblast has been described in detail by Florian (1928); in some situations the transition from one tissue to the other has been observed by us. In many areas the syncytiotrophoblast shows a brush border or ciliated border as described by Jung (1908), Herzog (1909), Stieve (1926), Florian (1928), Hill (1932), Jones & Brewer (1935), Brewer (1937; this author has also described and illustrated the development of this border), and Johnston (1941). In many areas in our specimen the border of the syncytiotrophoblast shows a foamy appearance similar to that described by Johnston (1941).

There is fairly general agreement that the syncytiotrophoblast absorbs nutritive material from the maternal blood (Florian, 1928; Wislocki & Streeter, 1938; and Johnston, 1941). Florian (1928) also suggests that it may form a protection to the cytotrophoblast. Jones & Brewer (1935) suggest that the brush border is associated with phagocytic activity, as does Bartelmez (quoted by Jones & Brewer). Streeter (1926) states that there is no ingestion of maternal stroma cells in the Miller ovum. Herzog (1909), on the other hand, thinks that a ciliated border is associated with the secretion of an enzyme which causes a coagulation necrosis of the tissue.

In some of the cells of the syncytiotrophoblast vacuoles are found similar to those described by Streeter (1920) in the Mateer embryo, Stieve (1926) in the Hugo embryo, and by Florian (1928). What the significance of these vacuoles may be Stieve is unable to say. Florian is of the opinion that they are evidence of degenerative changes. Grosser, in the discussion in Florian's paper (1928), points out that they are found in normal embryos. Politzer, on the other hand, regards them as pathological. We are unable to offer any definite opinion as to what their function may be, though possibly they are associated with absorption. Wislocki & Streeter (1938) believe that the large vacuoles (not quite similar to those described by us) in the macaque are a manifestation of absorptive activity of the trophoblast. The vacuoles in the syncytiotrophoblast should not be confused with the extensive vacuolation found in the degenerating masses of plasmoditrophoblast and described by many observers.

Outside the trophoblastic shell the proliferating syncytium sends out processes into the maternal tissue in the decidua capsularis, marginal and basal zones. Our observations confirm the view expressed by Florian (1928) that these apparently isolated masses seen in the sections are connected together by fine strands. Herzog describes the syncytial masses as penetrating the border zone and states that they have a tendency to break up into individual detached pieces. In the Shaw embryo the maternal tissue is actively invaded

by the strands as is shown by the destruction of uterine glands and blood vessels. It should be pointed out that in the region of the capsularis the syncytial masses frequently reach the lumen of the uterus (compare Brewer, 1937).

There is no evidence of the necrotic zone which is such an outstanding feature in early embryos, e.g. in Teacher-Bryce no. 1, and in Sch of von Möllendorff. A slight leucocytic infiltration round the implantation site is similar to that described by Jones & Brewer (1935). We find no evidence in support of the statement of Meyer (1924) that syncytial masses develop from the endothelium of the maternal capillaries.

At the areas of attachment of the cell columns to the maternal tissue it is very difficult to state exactly what is maternal and what is foetal tissue (cf. statement on p. 192). We were not so fortunate as Brewer (1937) to be able to use differential stains to demonstrate the reticulum in the maternal tissue. The foetal cells generally stain more intensely and are larger than the maternal cells.

Owing to the small size of the block of tissue removed with the embryo, and to the damage to the capsularis, it has not been possible to give a detailed account of the arrangement of the venous sinuses and arterioles in relationship with the implantation cavity.

A large basal sinus is situated in the basal part of the implantation cavity, as is typically found in most embryos. Capillaries are seen to open directly into the basal sinus, but no arterioles as shown by Stieve (1926) and Falkiner (1932) could be demonstrated to open into any part of the implantation cavity. Johnston (1941) states that a few capillaries in the decidua capsularis of the HR 1 embryo undoubtedly establish connexions with the intervillous spaces, but he thinks that the number is negligible.

A lateral sinus as described by Herzog (1909) and Stieve (1926) was found on one side of the present embryo; on approaching the marginal zone it divides into two, one branch running into the decidua capsularis, the other into the decidua parietalis.

There is a relatively small amount of blood in the intervillous spaces and in the surrounding glands compared with the amount present in the Peters, Teacher-Bryce II, Bi 1 (Florian, 1928), Werner (Stieve, 1936) and Brewer embryos, all of which are younger than the present specimen. In our opinion there are several explanations to account for the small amount of blood present: either the intervillous circulation has been established, or the large amount of blood of the earlier stages has been absorbed by the syncytiotrophoblast, or the amount of haemorrhage in some specimens is excessive. That the intervillous circulation has been established is the most probable explanation.

In the Falkiner (1932) and Frassi (1907) specimens there is an absence of blood in the implantation cavity. These, according to Falkiner, 'support the view that even as early as the second or third week, a definite circulation through the implantation cavity is effected'. In the HR 1 embryo, Johnston is of the opinion that the intervillous circulation has not been established and that

'the establishment of free circulation in the intervillous space at a later stage is not a gradual but rather a comparatively sudden process and ensures that increased entry of blood into the space, no matter how extensive, will not overtax the carrying capacity of the outlets'.

The point of entry of the ovum into the endometrium is visible between the lips of the decidua capsularis. It is closed by a plug of fibrin in the meshes of which are many red corpuscles and some leucocytes. The plug is continuous with a large blood clot which extends over the surface of the decidua capsularis superficial to the uterine epithelium. It is, therefore, a 'mushroom-shaped' coagulum similar to that described by Peters (1899, 1925) and, in the Yale embryo, by Ramsey (1938).

It will be in place here to draw attention to a confusion which has arisen in the use of the terms, (1) *operculum deciduae* (Latin: operculum—a small lid), (2) *Gewebspilz* (German: mushroom- or fungus-tissue), (3) *Verschlusscoagulum* (German-Latin form: occlusion-clot or closing-clot), (4) *Verschlusspfropf* (German: occlusion plug), (5) *Blutpfropf* (German: blood plug). We would suggest that the term operculum be limited to the flattened or dome-shaped head of the fungus- or mushroom-shaped structure; that the part occupying and closing the aperture of entry be called the 'occlusion- or closing-plug'; that the name 'mushroom-shaped plug' be used to denote the whole structure including the head and stem of the mushroom; and, in order that there can be no confusion, the expression 'fungoid tissue' be replaced by the term 'mycelioid tissue' in the description of the microscopical structure of the infiltrating plasmodial tissue, with reference to the resemblance of the latter to the continuous network of a mycelial growth. It should be mentioned, however, that the word 'fungoid' was originally used by Peters to denote a resemblance in form of the object under discussion to that of a fungus and not to the structural resemblance of the plasmodial growth in the plug to a mycelial growth.

The fate of the superficial clot appears to be variable: (1) it may be accidentally lost in the removing or preparation of the specimen, (2) it may under natural conditions become organized and persist for a time as a minute scar, or (3) it may become separated and shed off into the cavity of the uterus. It also appears to vary considerably in size and in degree of infiltration with plasmodium.

Covering the deep surface of the closing plug there is a layer of cytotrophoblastic tissue which is continuous with the trophoblastic shell. The structure of the closing plug is essentially similar to that found in the Herzog embryo. No trophoblastic elements are found in the closing plug in the Yale embryo. The closed canal in the Shaw embryo passes at an angle of approximately 45° to the surface of the capsularis and, therefore, obliquely as is the case in the Herzog, Teacher-Bryce I, Sch (von Möllendorff, 1921) and WO (von Möllendorff, 1925) embryos.

Remnants of the syncytium are present in the infiltrated blood clot, but not in the plug as described by Teacher (1924) in the Teacher-Bryce embryos I and II, and by von Möllendorff in the Sch embryo. In the Peters ovum and in the ovum of Linzenmeier (1914) the plug contains migrating cells from the trophoblast. Schlagenhaufer & Verocay (1916) found a plug comparable to that described by Teacher and regarded it as a method of closing the aperture in the decidua capsularis. In a young human embryo Dible & West (1941)

find that the aperture of entry is closed with fibrinous material similar to that in the implantation cavity.

The chorionic vesicle is no longer adherent to the capsularis at the point of entry, as is described in the Hugo (Stieve, 1926), the Falkiner, and the Edwards-Jones-Brewer (Brewer, 1937) embryos, but is completely freed from it. Teacher states that the operculum later becomes detached from the chorion as is, partially, the case in the present embryo.

A brief comparison of the present embryo with a corresponding stage of development of the macaque, as described by Wislocki & Streeter (1938), shows that the differentiation of the trophoblast, as pointed out by them, is essentially similar to that in man. They state that it is clear from perusal of the literature on early human embryos that the differentiation of the trophoblast in these imitates very closely the phases seen in the macaque.

Our embryo shows differentiation of the villi, trophoblastic shell, and penetration zone comparable to the macaque at about the 19th day of development. The villi in each case may be divided into three segments—a proximal, containing mesoderm, a distal, composed of loosely arranged cytotrophoblast, and an intermediate, which stains much more intensely than the distal segment. In the macaque, as in the human, the intervillous spaces are bounded by a layer of syncytial trophoblast which, according to Wislocki & Streeter, absorbs nutriment from the maternal corpuscles which have become swollen and irregular, and have lost their haemoglobin. They are also of the opinion that the products of the necrotic junctional zone supply nourishment to the trophoblastic shell.

The same difficulty is experienced in their beautifully graded macaque material, as in the human, in the junctional cellular zone, of differentiating accurately the precise boundary between degenerating maternal cells and the trophoblastic tissue. They state that 'there is no sharply differentiating stain so far developed to distinguish the two, and on morphology of nucleus and cytoplasm they cannot be separated with precision'. They further state that 'the cytotrophoblast in continuity with the cell columns extends deeply into the junctional zone spreading out below the intervillous space as "the trophoblastic shell"'.

SUMMARY

An account is given of the implantation of the 'Shaw' embryo which is approximately at the same stage of development as the Wa 17 embryo (Grosser, 1931); the estimated fertilization age of the 'Shaw' embryo would thus correspond to about the 17th or 18th day of development.

Villi are attached over the whole extent of the chorionic vesicle, being longest at the circumference.

A typical villus is differentiated into a basal part containing a mesodermal core, a distal part which consists of loosely arranged cell columns, and an intermediate part. The mesoderm of the basal segment contains angioblastic tissue some of which has advanced to the stage of capillary formation by the

fusion of intracellular vacuoles to form the lumina of capillary vessels. No blood corpuscles are as yet present in these capillaries, proving that, *in this situation*, the vessel walls are not formed by the union of flattened outer cells of blood islands, since no blood islands are present in the wall of the chorionic vesicle.

Frequent mitoses are present in the cytotrophoblastic cells at the bases of the cell columns, but are rare in the columns themselves, and it seems probable that the cellular activity of the cytotrophoblastic cells at the bases of the columns is sufficient to account for the growth of the cell columns. Appearances have been noted in the nuclei of the syncytiotrophoblast covering the villi, and the peripheral processes arising from these, which suggest amitotic divisions of the nuclei.

On the basal side of the chorionic vesicle the cell columns unite to form a distinct trophoblastic lamina, between the basal sinus and the intervillous space; towards the circumference of the chorionic vesicle and the decidua capsularis the cell columns are in continuity with the outer layer of the trophoblastic shell. The basal sinus communicates freely with the intervillous space by openings between the plates which form the cytotrophoblastic lamina.

Transitional stages between cytotrophoblast and syncytiotrophoblast of the villi have been observed, and in many places a definite 'brush border' is present and also minute vacuoles giving rise to the 'foamy' appearance described by Johnston. Large vacuoles and vacuoles of intermediate size are also present in the degenerating remnants of the plasmoditrophoblast.

Beyond the trophoblastic shell the proliferating peripheral syncytium sends out processes into the maternal tissue of the decidua capsularis, marginal and basal zones. These masses which appear, in sections, to be isolated, when traced through adjoining sections are frequently found to be connected and, although the connexions may be broken secondarily as a result of stretching or degeneration, it seems probable that the continuity, when present, is primary. Uterine blood vessels and uterine glands are invaded and destroyed by the peripheral syncytium.

Leucocytic infiltration, oedema, and degeneration of stroma cells, blood vessels and uterine glands are evident in places but no definite necrotic zone is visible. Some degenerating plasmodial tissue is found in the lumen of the uterus under cover of the free border of the operculum, between this and the uterine epithelium, as well as in the operculum itself.

A large basal sinus and a lateral sinus, in the marginal zone, are present. These are in free communication with the veins of the endometrium. Neither these nor the intervillous spaces contain much blood, and any red blood corpuscles or leucocytes which are found in recesses of the intervillous space are pale (lacking in haemoglobin), variable in size and partially disintegrated, giving the impression that they have been acted upon by some digestive ferment.

The point of entry of the ovum into the endometrium is closed by a plug of fibrin in the meshes of which are many red blood corpuscles and some leucocytes. The plug is continuous with a large blood clot which is flattened out over the surface of the decidua capsularis. The clot lies superficial to the uterine epithelium around the aperture of entry and on one side has blended with this, its edge being indeterminate, whereas, on the other side, the edge is free. On the deep surface of the plug, occupying the aperture of entry, there is a layer of cytotrophoblast which is continuous with the trophoblastic shell. The superficial fibrinous coagulum forming the head of the mushroom-shaped plug contains, in addition to degenerating red blood corpuscles and leucocytes, deeply stained pycnotic nuclei which appear to be the only recognizable remnants here of the plasmoditrophoblast, the body plasm of the original elements having disappeared or being only indistinctly visible.

Comparison of our embryo with a corresponding stage of development, about the 19th day, of the macaque embryo, as described by Wislocki & Streeter, affords additional confirmation of their conclusion that the differentiation of the trophoblast is essentially the same in man and the macaque.

We wish to acknowledge our indebtedness to the Publications Fund of the University of London for a grant towards part of the cost of the illustrations in our previous and present communications; and also for the cost of blocks for the coloured plate of our previous communication (Gladstone & Hamilton, 1941). We wish to thank Mr A. K. Maxwell for 'working up' the photographs of Pl. 1, fig. 1; Pl. 2, figs. 3, 4 and 5; Pl. 3, figs. 6 and 7; and Text-fig. 1. We wish also to thank our technicians, Messrs Westwood and Park, for their invaluable assistance.

ABBREVIATIONS USED IN THE FIGURES

ANG.	Angioblast.	*FB.*	Fibrin.
C. CY. TR.	Central cytotrophoblast (Langhan's Layer).	*IMP. C.*	Implantation cavity.
		OP.	Operculum.
BAS. SIN.	Basal sinus.	*PL. TR.*	Plasmoditrophoblast.
C. COL.	Cytotrophoblastic cell column.	*PR. SYN.*	Peripheral syncytium.
CY. PL.	Cytotrophoblastic plate(s).	*PR. TR.*	Primary plasmodium.
CY. TR.	Cytotrophoblast.	*SYN. TR.*	Syncytiotrophoblast.
D. BAS.	Decidua basalis.	*UT. EP.*	Uterine epithelium.
D. CAPS.	Decidua capsularis.	*V.L.*	Vacuole (large).
DEC. C.	Decidua cell(s).	*V.S.*	Vacuole(s) (small).
D. GL. C.	Degenerating gland cells.	*V. SYN. TR.*	Villous syncytiotrophoblast.
END.	Endothelium.		

REFERENCES

Brewer, J. I. (1937). *Amer. J. Anat.* **61**, 429.

Bryce, T. H. (1924). *Trans. Roy. Soc. Edinb.* **53**, 533.

Bryce, T. H. & Teacher, J. H. (1908). *Contributions to the Study of the Early Development and Imbedding of the Human Ovum.* Glasgow: Maclehouse.

Dible, J. H. & West, C. M. (1941). *J. Anat., Lond.*, **75**, 269.

FALKINER, N. M. (1932). *J. Obstet. Gynaec.* **39**, 471.
FLORIAN, J. (1928). *Anat. Anz.* Erg. **66**, 211.
FRASSI, L. (1907). *Arch. mikr. Anat.* **70**, 492.
GLADSTONE, R. J. & HAMILTON, W. J. (1941). *J. Anat., Lond.*, **76**, 9.
GROSSER, O. (1927). *Frühentwicklung, Eihautbildung und Placentation des Menschen und der Säugetiere.* Munchen: Bergmann.
—— (1931). *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **94**, 275.
HERZOG, M. (1909). *Amer. J. Anat.* **9**, 361.
HILL, J. P. (1932). *Philos. Trans.* B, **221**, 45.
JOHNSTON, T. B. (1941). *J. Anat., Lond.*, **75**, 153.
JONES, H. O. & BREWER, J. I. (1935). *Surg. Gynec. Obstet.* **60**, 657.
JUNG, P. (1908). *Beitrage zur frühesten Eieinbettung beim menschlichen Weibe.* Berlin: Karger.
KINDRED, J. E. (1933). *Amer. J. Anat.* **53**, 221.
LINZENMEIER, G. (1914). *Arch. Gynäk.* **102**, 1.
MEYER, P. (1924). *Arch. Gynäk.* **122**, 38.
MÖLLENDORFF, W. VON (1921). *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **62**, 406.
—— (1925). *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **76**, 16.
PETERS, H. (1899). *Die Einbettung des Menschlichen Eies.* Leipzig und Wein.
—— (1925). *Arch. Gynäk.* **124**, 625.
RAMSEY, E. M. (1938). *Contr. Embryol. Carneg. Instn*, **27**, 67.
SCHLAGENHAUFER, F. & VEROCAY, F. (1916). *Arch. Gynäk.* **105**, 151.
STIEVE, H. (1926). *Z. mikr. Anat.* **7**, 295.
—— (1936). *Z. mikr. Anat.* **40**, 281.
STREETER, G. L. (1920). *Contr. Embryol. Carneg. Instn*, **9**, 389.
—— (1926). *Contr. Embryol. Carneg. Instn*, **18**, 31.
TEACHER, J. H. (1924). *J. Obstet. Gynaec.* **31**, 166.
WISLOCKI, G. B. & STREETER, G. L. (1938). *Contr. Embryol. Carneg. Instn*, **27**, 1.

EXPLANATION OF PLATES 1–4

PLATE 1

Fig. 1. General view showing the relations of the embryonic rudiment and chorionic vesicle. The decidua basalis and a detached fragment of the decidua capsularis are also seen. The latter is traversed by the occlusion plug, and a part of the operculum is seen. The photograph was obtained from two original photographic prints which were pieced together and rephotographed so as to obtain a complete picture. An arrow indicates what we believe to be the point of entry. The interrupted line indicates the line of junction between the separate photographs. ×*c.* 15.

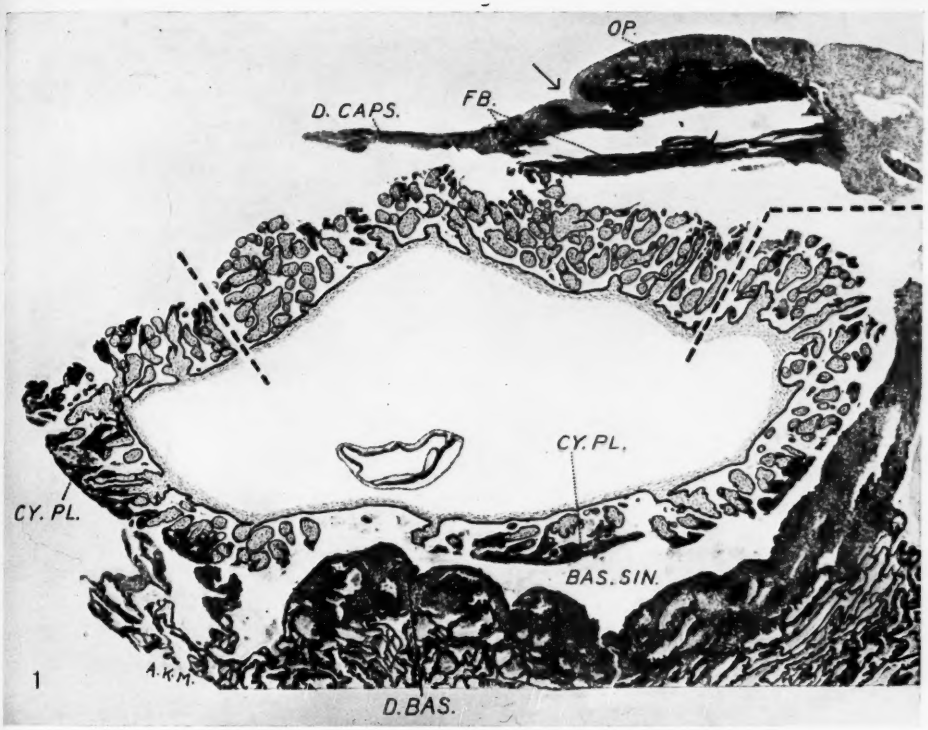
Fig. 2. Section through the wall of the chorionic vesicle and a part of the decidua basalis, showing the general relations of the villi, cytotrophoblastic plates and basal sinus. ×*c.* 140.

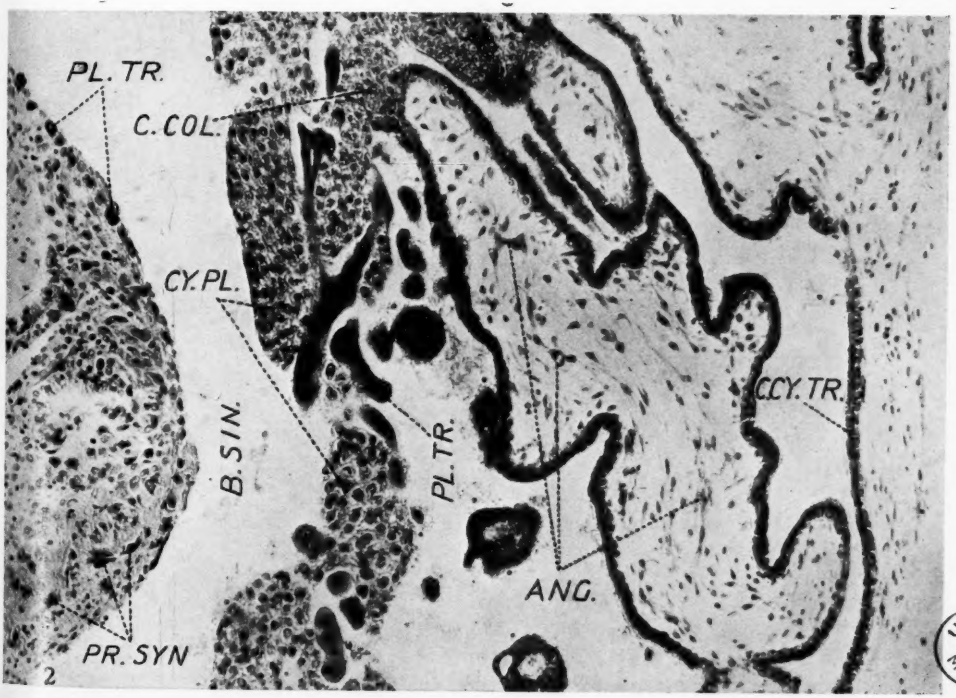
PLATE 2

Fig. 3. Transverse section through a typical villus showing the villous syncytiotrophoblast. Note large and small vacuoles and the 'brush border' on the bottom left-hand side of the figure. The nuclei are smaller than those of the cytotrophoblast and tend to be disposed in pairs which are in contact or close together and are approximately of the same size. A conspicuous single nucleolus, or two true nucleoli, are present, more especially at the ends of a row of nuclei, suggesting amitotic division. ×*c.* 550.

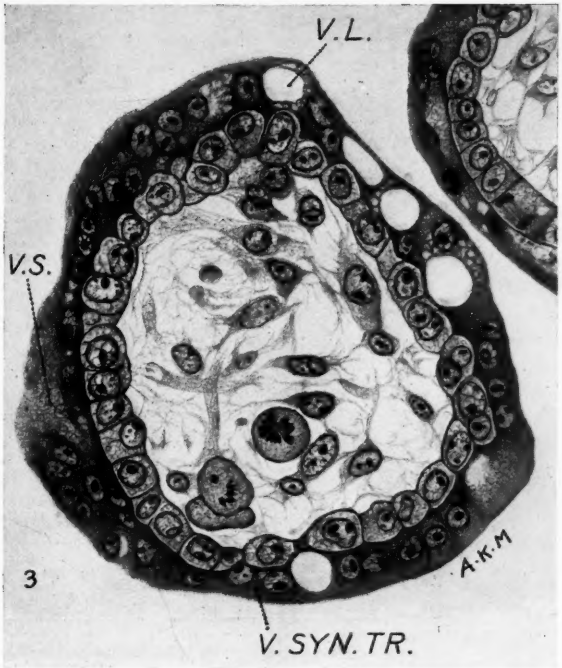
Fig. 4. Typical 'brush border' on the syncytiotrophoblast of a villus. ×*c.* 550.

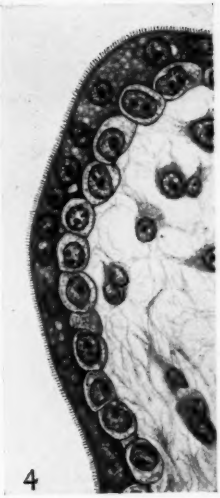
Fig. 5. Typical appearance of the cells of the cytotrophoblastic columns, showing bare areas and areas covered by syncytiotrophoblast. To the left and above in the figure are columns and masses which are probably remnants of the plasmoditrophoblast. ×*c.* 550.

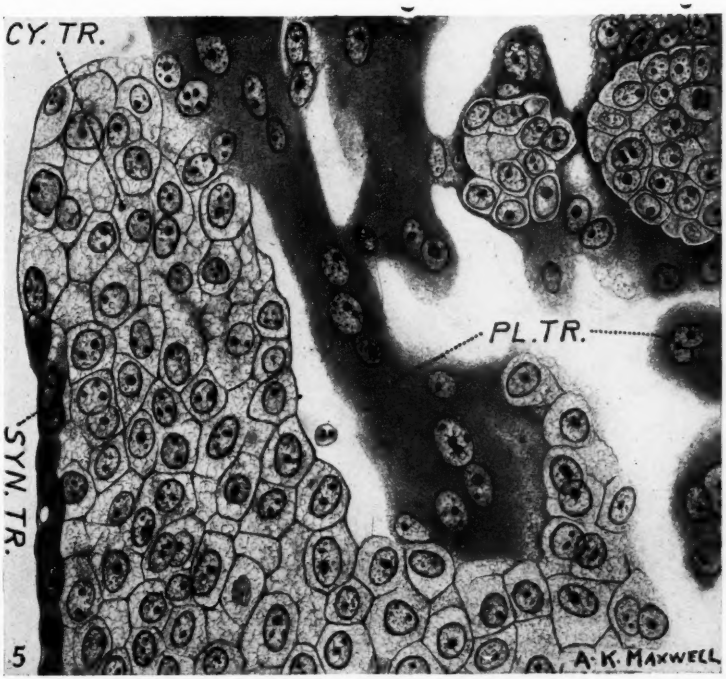

OP.
D. CAPS.
FB.
CY. PL.
CY. PL.
BAS. SIN.
A. K. M.
D. BAS.
1


PL. TR.
C. COL.
CY. PL.
B. SIN.
PL. TR.
CCY. TR.
ANG.
PR. SYN
2

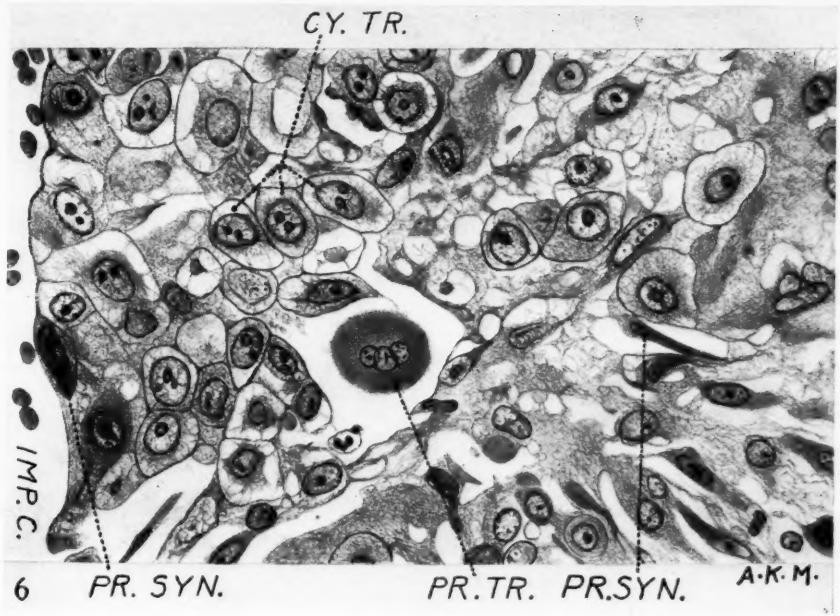
UNIV. OF MICH.

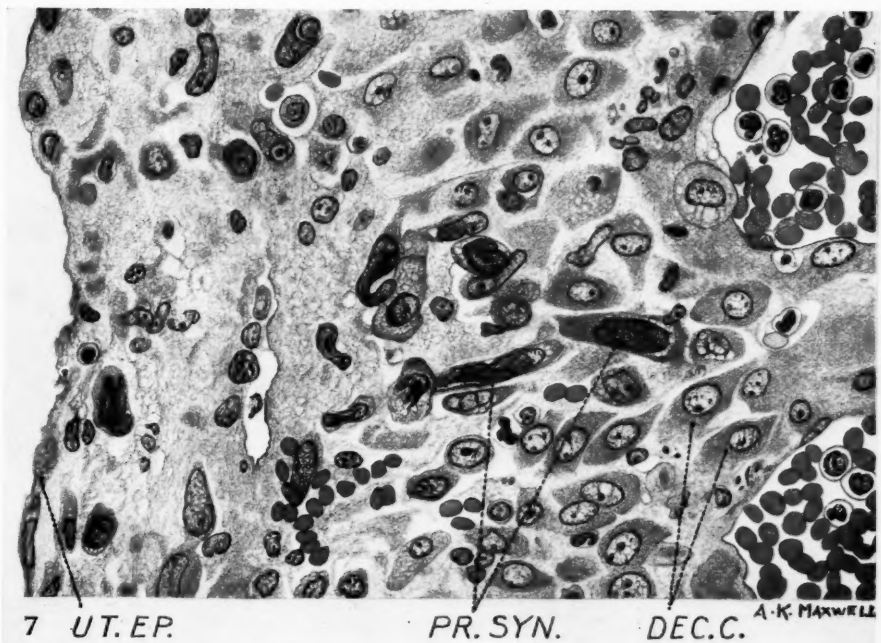






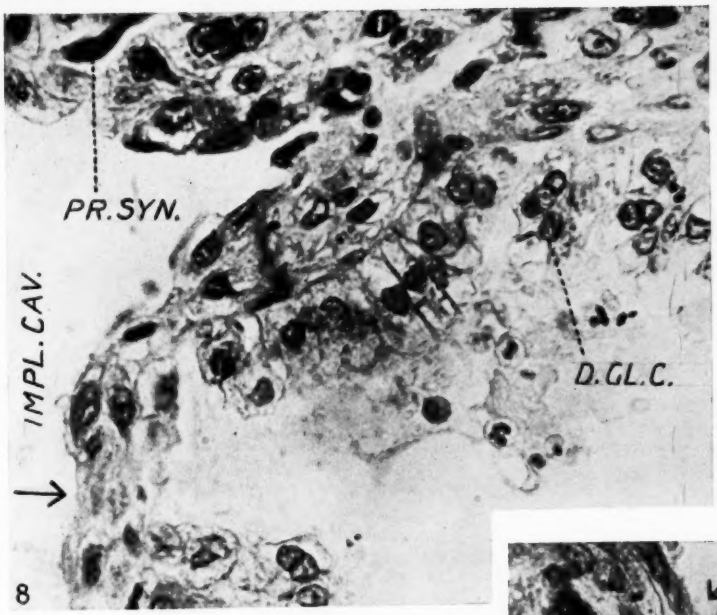
UNIV. OF MICH.

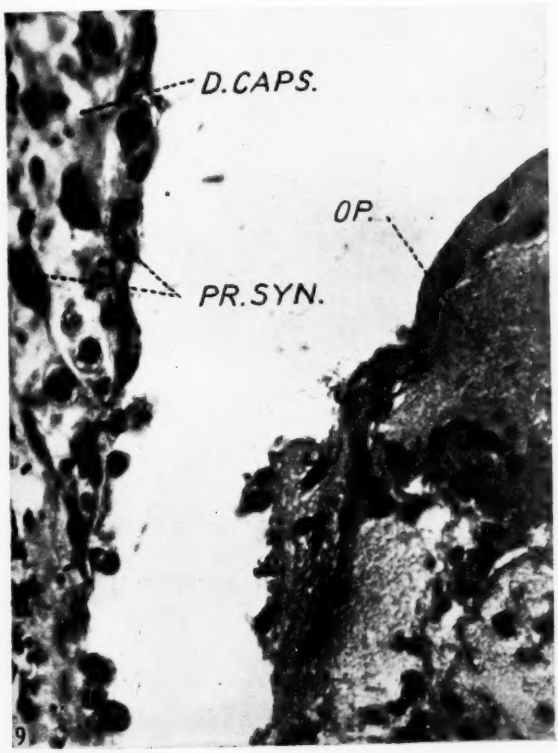


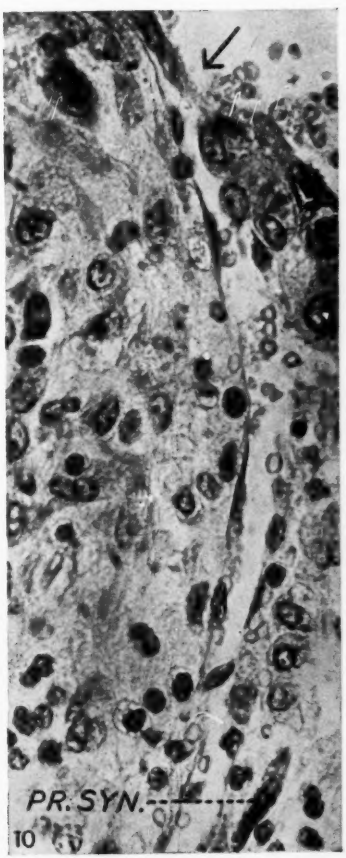


HAMILTON AND GLADSTONE—PRESOMITE HUMAN EMBRYO

UNIV. OF MICH.







UNIV. OF MICH.

PLATE 3

Fig. 6. Junctional penetration zone at the attachment of the cell columns to the endometrium. Note the invasion of the maternal tissue by both cytotrophoblast and peripheral syncytium. × *c.* 550.

Fig. 7. Showing the invasion of the decidua capsularis by peripheral syncytium. × *c.* 550.

PLATE 4

Fig. 8. Uterine gland opening into the implantation cavity at the point indicated by the arrow. The glandular epithelium is degenerating and leucocytes are visible in the cell detritus occupying the lumen, and in the interglandular tissue which also contains strands of peripheral syncytium. × *c.* 550.

Fig. 9. Showing the free edge of the operculum where it overlaps the decidua capsularis and covers remnants of the uterine epithelium which are degenerating and discontinuous. The operculum consists of a fibrinoid matrix containing degenerated plasmodial elements, red blood corpuscles and leucocytes. Peripheral syncytial elements are also seen in the stroma of the decidua capsularis which also contains many lymphocytes and leucocytes. × *c.* 550.

Fig. 10. Showing a capillary opening into the basal sinus at the point indicated by an arrow. Above the capillary is a strand of proliferating syncytium. × *c.* 550.

LIVING EGGS OF THE GOLDEN HAMSTER (*CRICETUS AURATUS*)

By D. M. SAMUEL and W. J. HAMILTON

Anatomy Department, St Bartholomew's Hospital Medical College, London

Some specimens of the golden hamster, *Cricetus auratus*, first described by Waterhouse in 1839, were obtained in Syria in 1930 and reported by Aharoni in 1932. Two pairs were brought to England in 1933 and were used for breeding by Prof. E. Hindle, of Glasgow, who later published notes on their breeding and growth (Bruce & Hindle, 1934). These animals are remarkable for the shortness of their gestation period, this being, on the average, only 16 days. Biological studies have only recently been made on the species; its reproductive cycle has been described by Deanesley (1938).

We are very much indebted to Prof. Hindle who supplied us with the original breeding stock for these investigations, and to the Institute of Animal Genetics, Edinburgh, who provided additional animals.

The present communication is an account of the early development of the golden hamster, and deals only with living eggs up to, and including, the eight-cell stage of development.

TECHNIQUE

A review of the methods used for procuring living mammalian eggs is given by Squier (1932). In the golden hamster the uterine tube is long, narrow and tortuous, measuring in the fresh state approximately 33 mm. in length and 0·4 mm. in external diameter. The uterine horns are separate, the two canals fusing within the cervix.

The tube was first dissected away from the horn and ovary and then unravelled, using a low-power binocular dissecting microscope. An attempt was first made to flush out the tube with saline with the help of fine hypodermic needles and finely drawn out glass pipettes. This method we discarded as unsatisfactory owing to the small diameter of the uterine tube. We then resorted to a method originally mentioned by Cruikshank (1797) and since used by many investigators. The unravelled tube is straightened and divided into three approximately equal segments. From each of these the epithelium, with any contained eggs, is expelled by gradual firm pressure with some instrument such as a blunt needle along the length of the tube, which is further subdivided if necessary. This method gives an indication of the position of the eggs at the different developmental stages. Eggs secured by this means appear normal in every respect and are easily recognizable owing to the refractile nature of the zona pellucida. Some eggs are, however, occasionally lost or damaged.

Individual eggs are then pipetted through several changes of normal saline to remove debris, this procedure being carried out as rapidly as possible.

The eggs were photographed in normal saline on a Vickers projection microscope which, having the optical system inverted, is well suited to this type of work. Photographs were taken at varying magnifications and at different levels in the egg. We find that, in general, a low-power objective with a high secondary magnification gives the best general picture of the whole egg.

Accurate timing of insemination is difficult, since only on one occasion have we observed mating in the daytime. Our animals have not been watched at night, but Deanesly (1938) states that mating occurs usually after 10 p.m. We have found that the simplest method of determining the date of insemination is to take daily vaginal smears and to examine them for the presence of spermatozoa. For this reason the ages of the eggs given in this paper are estimated by reference to the day sperms were found in the vaginal smear or the first or second day following this. If midnight is taken as the average time of insemination, the times in hours after insemination would be approximately 10, 34 and 58, with 64 hr. for those taken on the afternoon of the 'second day'.

DESCRIPTION

Unsegmented unfertilized eggs. The egg illustrated in Pl. 1, fig. 1 was recovered from the middle third of the uterine tube of an unmated animal whose oestrous cycle had not been checked. Two eggs were recovered, the second being oval.

There is a thick zona pellucida which has a homogeneous glassy appearance. The vitellus appears granular, with some moderate-sized aggregations of granules, and is translucent owing to the absence of the fatty globules which are a feature of so many mammalian eggs. No polarity of the cytoplasm is observed and a proper perivitelline space is not formed, though small gaps are seen between the vitellus and the zona, possibly due to shrinkage. No polar bodies are seen. By appropriate focusing it is possible to see the nucleus. Measurements of this egg, with those of the others, are given in Table 1.

Unsegmented fertilized eggs. Up to the present time we have not investigated fertilized eggs before the second polar bodies have been given off. Unsegmented eggs are easily obtained by killing the animal on the day that sperms are found in the vaginal smear. Two such eggs are shown in Pl. 1, figs. 2 and 3. None of them show any trace of remaining cells of the corona radiata. The vitellus no longer fills the cavity within the zona pellucida, and there is, therefore, a definite perivitelline space. Two nuclear masses can be seen in these eggs; so it is presumed that fertilization must have taken place. Two polar bodies are observed to be free in the perivitelline space; they bear no constant relationship to each other (Pl. 1, figs. 2, 3). Eggs with three polar bodies have not been observed.

Two-cell stage eggs. These, again, are easily obtainable by killing the animal

on the morning of the first day after sperms are found in the vaginal smear. The eggs have now reached two-thirds of the way along the uterine tube.

The blastomeres show no polarity of the vitellus and have fairly obvious centrally placed nuclei. The cells usually show flattening at the surfaces in contact with each other, but in the egg illustrated (Pl. 1, fig. 4) they are nearly spherical. For the most part division is apparently equal, but in some cases one cell is seen to be distinctly the larger. One or two polar bodies may be seen in the perivitelline space, usually between the zona pellucida and the intercellular sulcus.

Four-cell stage eggs. These are obtained by killing the animal on the morning of the second day after sperms are found in the vaginal smear. They may be in the distal third of the tube, at the tubo-uterine isthmus, or even in the uterine horn.

The cells of the two-cell stage have apparently divided at right angles to each other, for the four-cell stage shows the typical cross-arrangement of the

Table 1

	O.D.Z. μ	I.D.Z. μ	D.V. μ	Approx. vol. of intrazonal cavity cu. μ	Volume of vitellus cu. μ
One-cell stage (unfertilized)	114	94	94	435,000	435,000
One-cell stage (fertilized)	118	94	71·4	435,000	191,000
Two-cell stage	117	97	—	478,000	—
Four-cell stage	119	102	—	555,000	—
Eight-cell stage	124	103	—	570,000	—

O.D.Z. outer diameter of zona; I.D.Z. inner diameter of zona; D.V. diameter of vitellus.

blastomeres (Pl. 1, fig. 5). The cells are almost spherical, and centrally placed nuclei are visible. The cells may show slight differences in size, and their total volume does not approach that of the intrazonal space (see Table 1). The polar bodies are still present, and have no characteristic positions.

Eight-cell stage eggs. These may be recovered either from the isthmus of the tube or from the uterine horn. They are to be obtained later during the second day after sperms are found in the smear (about 66 hr. after copulation). The cells are spherical and show slight differences in size (Pl. 1, fig. 6). Polar bodies, or their remains, are still often readily identifiable.

Measurements of the eggs. Scanty material has been available for some of the stages; as a general indication, therefore, two typical eggs have been chosen for each type. We have not been able to prevent evaporation entirely, and volumetric determinations of the vitellus may thus be somewhat underestimated. These data, therefore, are only approximate, and more detailed figures will be given in a further communication.

COMMENTS AND COMPARISONS

There is now an extensive literature dealing with living mammalian eggs. Reviews have been published by Lewis & Wright (1935) and Pincus (1936). Hartman (1929) has reviewed the comparative sizes of mammalian eggs. It is our intention to deal only with the eggs of rodents.

In size the egg of the golden hamster closely approximates to that of the mouse and rat as given by Macdonald & Long (1934) and Lewis & Wright (1935). The one-cell egg after fertilization has been taken as the basis for the comparison in Table 2.

Table 2

	O.D.Z. μ	I.D.Z. μ	D.V. μ	Volume of vitellus cu. μ
Mouse	113	87	71·6	192,000
Rat	108	80	76	230,000
Golden hamster	118	94	71·4	191,000

These measurements are smaller than those for the guinea-pig, D.V. approximately 80μ (Squier, 1932), and very much smaller than those for the rabbit, D.V. approximately 122μ (from illustrations by Gregory, 1930).

The formation of a perivitelline space subsequent to fertilization is in general agreement with published observations on mammalian eggs. This space allows the polar bodies freedom of movement, and their position is therefore no indication of their site of origin.

The vitellus in rodents is, in general, free from fatty globules, with the exception of the guinea-pig in which small globules of lipoid material are evenly distributed throughout the vitellus except for the peripheral zone. These globules obscure the nucleus to a large extent (Squier, 1932). The appearances of the vitelli in Pl. 1 do not give an adequate representation of the appearances of the eggs under the microscope. Much of the granular material is highly refractile, appearing as adjacent dark and light spots which change their positions with slight alterations of focusing.

In the mouse, a small amount of granular and globular yolk material has been described as being evenly distributed throughout the vitellus of unicellular eggs after the formation of the second polar body (Lewis & Wright, 1935). A similar condition in the rabbit is reported by Gregory (1930) and Pincus (1930). In the rat the cytoplasm is described by Macdonald & Long (1934) as being clear and transparent, with fine granules which are evenly distributed throughout the vitellus of the unfertilized egg, but which are grouped into aggregations after fertilization.

The finding of eight-cell stage eggs typically in the uterine horn is dissimilar from the condition in the rat and mouse where uterine eggs are at the morula or blastocyst stage. It is, however, similar to the condition in the guinea-pig in which, incidentally, a single four-cell stage egg has been found in the uterine horn, a condition we have occasionally observed in this hamster.

SUMMARY

An account is given of living eggs of the golden hamster and comparisons are made with eggs of other rodents.

REFERENCES

Aharoni, B. (1932). *Z. Säugetierk.* **7**, 166.
Bruce, H. M. & Hindle, E. (1934). *Proc. Zool. Soc. Lond.* **104**, 361.
Cruikshank, W. (1797). *Philos. Trans.* **87**, 197.
Deanesly, R. (1938). *Proc. Zool. Soc. Lond.* **108**, 31.
Gregory, P. W. (1930). *Contr. Embryol. Carneg. Instn*, **21**, 143.
Hartman, C. G. (1929). *Quart. Rev. Biol.* **4**, 373.
Lewis, W. H. & Wright, E. S. (1935). *Contr. Embryol. Carneg. Instn*, **25**, 115.
Macdonald, E. & Long, J. A. (1934). *Amer. J. Anat.* **55**, 343.
Pincus, G. (1930). *Proc. Roy. Soc.* B, **107**, 132.
—— (1936). *The Eggs of Mammals.* New York: Macmillan.
Squier, R. R. (1932). *Contr. Embryol. Carneg. Instn*, **23**, 225.
Waterhouse, G. R. (1839). *Proc. Zool. Soc. Lond.* **7**, 57.

EXPLANATION OF PLATE 1

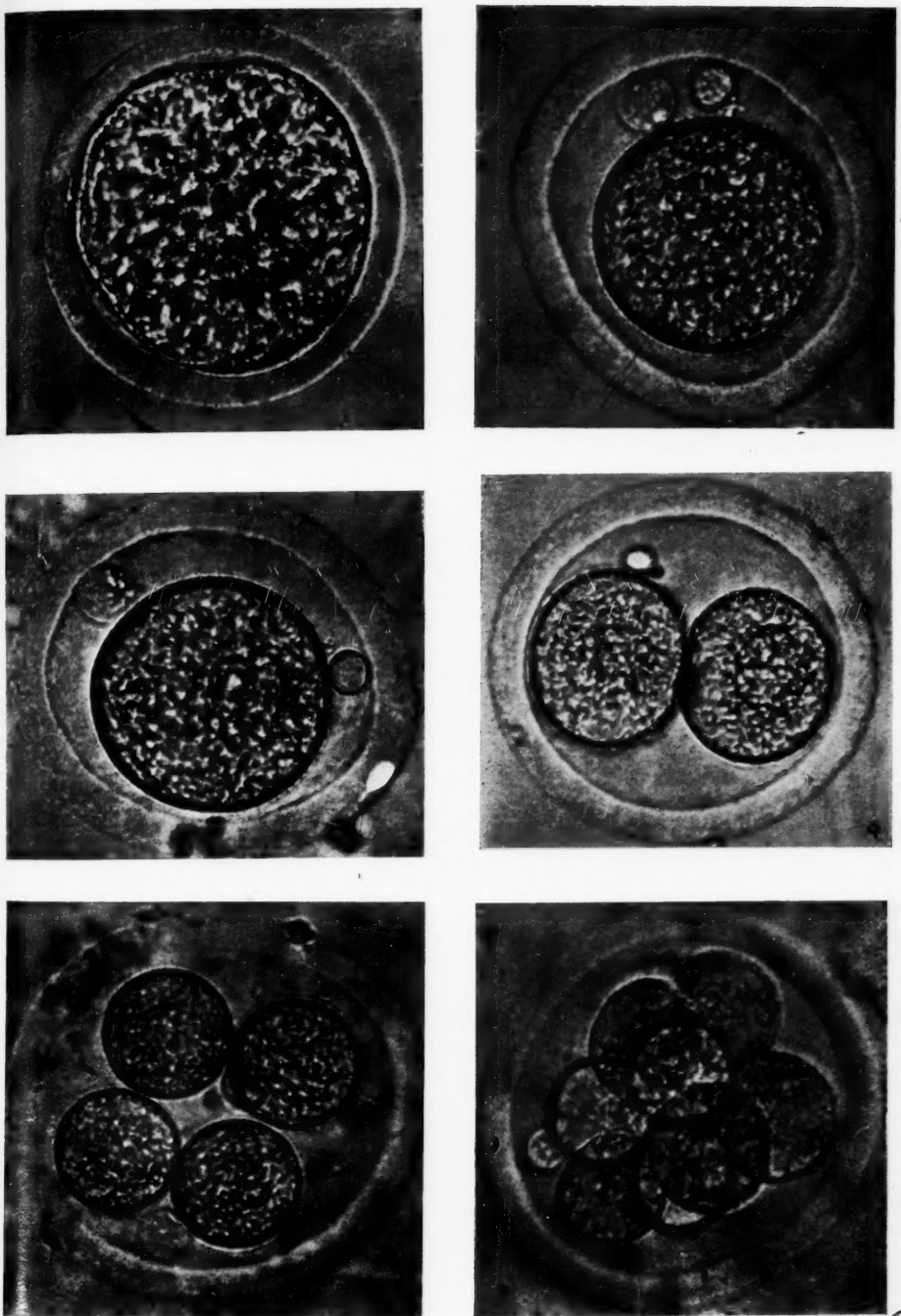
Fig. 1. Unfertilized egg of the golden hamster. No definite perivitelline space is shown. Granules show aggregation. ×490.

Figs. 2 and 3. Fertilized one-cell eggs. Two polar bodies are together in Fig. 2 but are well separated in Fig. 3. ×490.

Fig. 4. Two-cell egg with single polar body. ×490.

Fig. 5. Showing four-cell egg. Two polar bodies are still present. ×490.

Fig. 6. Eight-cell egg. One polar body still visible. ×490.



SAMUEL AND HAMILTON—LIVING EGGS OF THE GOLDEN HAMSTER (*CRICETUS AURATUS*)

UNIV. OF MICH.

THE EFFECT OF THE METHOD OF ADMINISTRATION ON THE MORPHOGENETIC ACTIONS OF PROGESTERONE

By HELEN WINTER

Department of Anatomy, McGill University, Montreal

It has recently been shown that if progesterone is administered intraperitoneally in sufficiently high doses it causes deep anaesthesia, while subcutaneous administration of the same or even of still higher doses exerts no such effect. This has been attributed to the greater rapidity with which it is absorbed from the peritoneum than from the subcutaneous tissue. The fact that the rate of absorption plays a particularly important role in determining the anaesthetic potency of progesterone is well shown furthermore by the observation that intravenous administration of this compound in propylene glycol causes almost immediate anaesthesia (Selye, 1941*a*).

It appeared of interest in this connexion to establish whether there is any noticeable difference between the morphogenetic actions of intraperitoneally and intravenously administered progesterone. Since some of the actions of this hormone are also significantly influenced by the pituitary (Selye, 1940; Selye & Bassett, 1940) it was deemed of value to reinvestigate, in connexion with the above-mentioned problem, the difference in the response of the intact and hypophysectomized progesterone-treated animal.

EXPERIMENTAL

Forty female albino rats, having an average weight of 62 g. (range 56–71 g.), were divided into the following five groups of eight rats each: (1) untreated, (2) subcutaneously injected with progesterone, (3) intraperitoneally injected with progesterone, (4) hypophysectomized and subcutaneously injected with progesterone, (5) not injected, hypophysectomized. The rats were treated every 2 days, first with a dose of 3 mg./day, and this amount was gradually increased up to 10 mg./day. This gradual increase in the amount of progesterone given was essential because the animals receiving the hormone intraperitoneally were definitely anaesthetized after each injection during the first few days and, as previous experiments have shown, the daily dose must be raised gradually in order to obtain adaptation to the narcotic effect of this compound (Selye, 1941*b*). The progesterone was dissolved in peanut oil in a concentration of 40 mg./ml. Since it has been shown by Selye & Bassett (1940) that progesterone exerts a marked diuretic action both in the intact and even more so in the hypophysectomized rat, it was also decided to follow the urine output of the experimental animals. In order to make this possible with a reasonable degree

of accuracy, each rat was placed in a special metabolism cage of a type designed in this laboratory to make possible complete separation of food, faeces and urine (Selye *et al.* 1941). The urine volume was measured daily.

The results of this experiment are summarized in Table 1 and Fig. 1, in which the average organ weights and urine volume are registered as well as the percentage deviation of these values from those of the intact controls. It is readily seen that subcutaneous injection of progesterone causes marked adrenal atrophy in the intact rat, which confirms previous observations (Selye & McKeown, 1935). On the other hand, if the same amount of the hormone is administered intraperitoneally it causes no significant change in adrenal weight; indeed, the adrenals in this group are slightly larger than those of untreated controls. In the hypophysectomized animal progesterone failed to influence adrenal weight. The ovarian and thymus atrophy which is usually

Table 1. *Effect of progesterone on the weight of various organs and diuresis in the intact and hypophysectomized rat*

	Controls	Progesterone s.c.*	Progesterone i.p.*	Hypophysectomized + progesterone s.c.*	Hypophysectomized
Body weight in g.	77·0	80·0	83·0	63·0	58·0
Adrenal weight in mg.	22·1	16·3	23·7	6·0	5·8
Thymus weight in mg.	173·0	110·0	150·0	48·3	122·0
Ovary weight in mg.	14·6	5·8	7·3	4·15	3·5
Uterus weight in mg.	55·0	203·0	145·0	167·0	19·9
Urine volume in ml. average/day during experiment	3·8	4·5	5·2	8·7	4·1
Percentage deviation from controls	0	+18·0	+37·0	+129·0	+7·0

* s.c. = subcutaneous. i.p. = intraperitoneal.

caused by subcutaneous progesterone administration was well marked in this series and, although even intraperitoneal administration of the hormone elicited a slight decrease in the average weight of these organs, the atrophy was by no means as pronounced as after subcutaneous injection. Conversely, the uterus, which increases in size under the influence of progesterone, shows a more pronounced hypertrophy following subcutaneous than following intraperitoneal injections. This uterine hypertrophy elicited by progesterone in animals not pretreated with oestrin was first described by Selye (1940), who demonstrated, furthermore, that it may also occur in the absence of the pituitary. The present experimental series shows that owing to the marked atrophy of the uterus which develops following ablation of the pituitary the metrotropic effect of the hormone is even more pronounced in the hypophysectomized than in the intact animal. It is of special interest in this connexion that, although the ovaries and adrenals of the hypophysectomized animal are not modified by progesterone treatment, the thymus-weight-decreasing effect

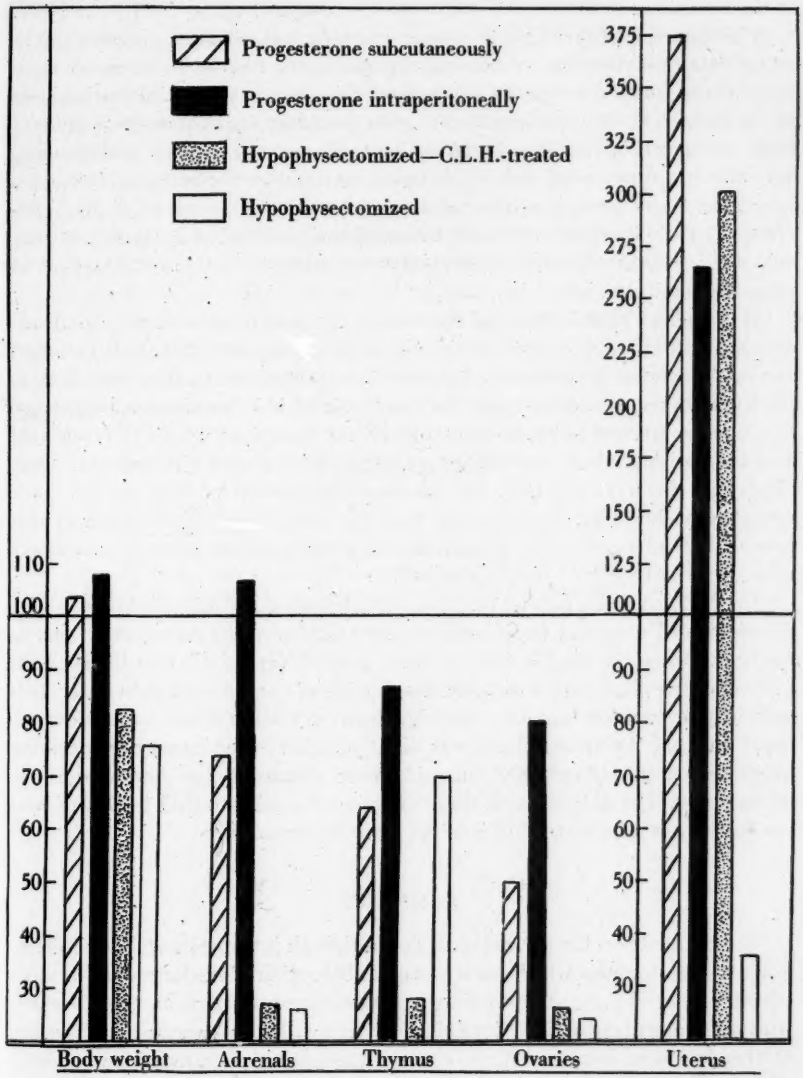


Fig. 1. Effect of subcutaneous and intraperitoneal progesterone administration on the weights of various organs. In this curve the average organ weights are given in a manner to indicate the percentage deviation from the untreated control values which are taken to be 100. Since the uterine weights are very high in proportion to those of other organs, the scale is changed above the base level of 100 for the columns representing the uterus.

of the hormone is likewise more pronounced in the absence than in the presence of the pituitary.

The diuretic action of progesterone was only insignificantly greater in the intact rats receiving the hormone intraperitoneally than in those receiving it by subcutaneous injection. In this respect the method of administration does not appear to be of great importance. On the other hand, it is quite evident from the figures given in Table 1, which represent the average daily output over the entire experimental series, that the diuretic effect of progesterone is very much more pronounced in the absence than in the presence of the hypophysis. This is in agreement with the assumption of Selye & Bassett (1940), who believe that some pituitary factor counteracts the diuretic action of progesterone in the intact animal.

Histological examination of the mammary glands showed no significant development of the acinar system in any of the groups except that of the intact animals receiving progesterone by the subcutaneous route. The fact that in the hypophysectomized animals the same dose of this hormone administered in the same manner failed to cause significant mammary gland development is not surprising, since previous experiments have shown the important role played by the hypophysis in the mammotropic action of this steroid. It is noteworthy, however, in connexion with the main problem discussed in this paper, that intraperitoneal administration proved almost entirely ineffective even in the presence of the hypophysis.

In order to obtain further evidence corroborating the data described above we performed a second experiment under practically the same conditions as the first. Since the results were in every respect very similar to those of the first series, we shall omit a detailed description of the relevant data here. It is sufficient to mention that the only significant deviation from the first experiment was that the urine volume was slightly higher in the intact rats receiving progesterone subcutaneously than in those obtaining the hormone intraperitoneally. The data of both these experiments indicate that in this respect the method of administration is of no great importance.

SUMMARY

Experiments on the rat indicate that although intraperitoneal administration of progesterone—which leads to rapid absorption of the hormone—is more effective in producing anaesthesia than subcutaneous injection, the reverse is true of the morphogenetic actions of progesterone. Thus subcutaneous injection of this hormone is more effective than intraperitoneal administration with regard to its ability to cause atrophy of the adrenal cortex, thymus and ovaries or hypertrophy of the uterus.

The diuretic action of progesterone is apparently independent of the method of administration under the conditions of the present experimental series.

It is noteworthy that in the absence of the hypophysis the thymus atrophy,

uterine hypertrophy, and diuresis caused by progesterone are much more pronounced than in the presence of this gland.

Histological examination showed, furthermore, that the mammotropic effect of progesterone is much more pronounced in the case of subcutaneous administration than it is after intraperitoneal injection. Thus of all the progesterone effects so far examined, only the anaesthetic action proved to be more evident following intraperitoneal administration of the compound than following its injection into tissues from which it is presumably more slowly absorbed.

These experiments furnish additional evidence emphasizing the importance of the method of administration for the action of hormones.

The expenses of this investigation were defrayed through a grant received from the Blanche E. Hutchinson Fund of McGill University. The progesterone was kindly donated by Dr Gregory Stragnell and Dr Erwin Schwenk of the Schering Corporation, Bloomfield, New Jersey.

REFERENCES

SELYE, H. (1940). *Anat. Rec.* **78**, 253.
—— (1941*a*). *Proc. Soc. exp. Biol., N.Y.*, **46**, 116.
—— (1941*b*). *J. Immunol.* (in the Press).
SELYE, H. & BASSETT, L. (1940). *Proc. Soc. exp. Biol., N.Y.*, **44**, 502.
SELYE, H., BASSETT, L. & NIELSEN, K. (1941). *J. Lab. Clin. Med.* (in the Press).
SELYE, H. & MCKEOWN, T. (1935). *Proc. Roy. Soc.* B, **119**, 1.

ENTERECTOMY AND THE BLOOD LYMPHOCYTES

By J. M. YOFFEY

Department of Anatomy, University College of South Wales and Monmouthshire, Cardiff

During health the white cells of the blood are maintained at a fairly constant level. For the maintenance of this level Naegeli (1931) postulated a hormonal control, though a review such as that of Hoff (1934) shows that we are still ignorant of the nature of the control mechanism. This ignorance applies to all the white cells, including the lymphocytes, even though in their case we possess information not available for the others, namely, quantitative data on the number entering the blood (Yoffey, 1936). If we also knew where the lymphocytes left the blood, we might be able to launch a direct experimental attack on the control of the blood lymphocyte level; but the fate of the blood lymphocyte is still unknown, though a number of hypotheses have been put forward.

Bunting & Huston (1921) suggested that the lymphocytes disappear from the blood by migrating into the lumen of the alimentary canal. If this is so, removal of a large part of the alimentary canal should interfere with the process of elimination, and the number of lymphocytes in the blood should increase. This was the working hypothesis upon which the following experiments were based. The results are inconclusive, but they are presented here since they illustrate well some of the complexities of the problem.

MATERIAL AND METHODS

The work was done on dogs, anaesthetized by a C.E. mixture followed by intravenous chloralosane (0·1 g./kg. body weight). Through a median abdominal incision the whole of the small intestine was removed distal to the duodenum, together with most of the large intestine. The cut ends of the intestine were closed. The main mesenteric vessels were tied in one ligature at the root of the mesentery, just before entering the glandula mesenterica magna—a large lymphoid mass lying at the root of the mesentery (sometimes referred to as the pancreas of Aselli). Where possible, glandula, intestine, and mesentery were removed in one mass. After closing the abdomen, a sample of blood was taken from the ear. Total and differential white cell counts were made, and in most cases the red cells were also counted. This was the first count, count *A*, performed immediately after the operation. It was followed, after a period varying from 2 to 3 hr., by a second count, count *B*. The animal was then killed and the abdomen reopened, in order to measure the volume of the peritoneal exudate.

RESULTS

The complete data for a typical experiment are given in Table 1. The essential data of the fifteen experiments are summarized in Table 2. Thus, to take the summarized data for animal no. 2: The interval between counts *A* and *B* was 2 hr. During that time the blood lymphocytes fell from 1970 to 1400 per cu. mm., a drop of 570. If count *A* is taken as 100 %, then count *B* is 71 % of this. The red cells rose from 6,030,000 to 6,710,000, and the neutrophils from 2170 to 13,150.

The percentage changes in the blood lymphocytes are presented separately in a graph (Fig. 1).

Table 1. *Protocol of a typical experiment*

Dog no. 14. 19. ii. 37. Weight 15·19 kg.

10.05 a.m. Anaesthetic begun.
10.25 a.m. Cannula tied in femoral vein and 1·5 g. of chloralosane given intravenously.
11.25 a.m. Enterectomy completed. 8 ft. of intestine removed in all, of which 5 in. were large intestine.
Blood count *A* made.
2.25 p.m. Blood count *B* made.
Animal killed. On opening abdomen 20·0 c.c. of blood-stained fluid found in peritoneal cavity.

DETAILS OF BLOOD COUNTS

	Count *A*	Count *B*
Red cells	8,560,000 per cu. mm.	8,640,000
White cells	12,170	22,300
	Differential	
Lymphocytes	2,550 (20·9 %)	1,450 (6·5 %)
Neutrophils	7,970 (65·5 %)	19,600 (88 %)

DISCUSSION

The experiments are open to some obvious criticisms. For example, was sufficient time allowed to elapse between counts *A* and *B* for any appreciable change in the blood lymphocytes to take place? In previous work (Yoffey, 1935) similar periods of time seemed to be adequate for the development of significant changes in the blood lymphocytes; but in view of the inconclusive results of the present series, experiments of longer duration are clearly indicated, supplemented if necessary by recovery experiments. Attention should also be paid to the blood lymphocytes in human beings who have recovered after extensive enterectomy.

Again, was sufficient intestine removed to subject to a fair test the hypothesis of the intestinal elimination of lymphocytes? After removal of jejunum, ileum, and most of the large intestine, the stomach and duodenum are probably the most important of the remaining portions of the alimentary canal from the point of view of cell elimination, especially as it has been shown (Loeper & Marchal, 1922) that the stomach may contain an appreciable number of white

cells, though according to the work just quoted these are chiefly granulocytes. But even though stomach and duodenum were left behind in the present experiments, the length of intestine removed has been such (for details see Table 2) that its removal might reasonably be expected to exert a significant influence.

Table 2. *Changes in blood cells in fifteen dogs following enterectomy*

No. of animal, and of count (whether *A* or *B*)	Blood lympho-cytes per cu. mm.	Percentage change in blood lympho-cytes	Blood neutro-phils per cu. mm.	Erythro-cytes in blood	Time between counts *A* and *B*	Absolute change in blood lympho-cytes per cu. mm. between counts *A* and *B*	Peri-toneal exudate in c.c.	Length of intestine removed
1 *A*	3,700	100	10,210	—	2 hr.	− 40	—	—
B	3,660	99	20,700					
2 *A*	1,970	100	2,170	6,030,000	2 hr.	− 570	—	—
B	1,400	71	13,150	6,710,000				
3 *A*	4,760	100	6,350	6,830,000	2 hr. 35 min.	−3,060	95	—
B	1,700	36	11,100	6,000,000				
4 *A*	1,880	100	12,660	7,200,000	2 hr. 25 min.	+ 10	—	6 ft. 9 in.
B	1,890	100·5	19,100	7,760,000				
5 *A*	2,830	100	9,300	6,060,000	2 hr. 20 min.	− 290	40	8 ft. 6 in.
B	2,540	90	27,900	6,160,000				
6 *A*	1,050	100	13,680	6,430,000	2 hr. 40 min.	+ 320	40	5 ft. 5 in.
B	1,370	130	33,700	5,920,000				
7 *A*	2,980	100	4,600	7,000,000	3 hr.	+1,320	4	8 ft.
B	4,300	144	25,230	6,850,000				
8 *A*	1,830	100	31,100	5,200,000	3 hr. 15 min.	+ 410	40	10 ft. 8 in.
B	2,240	122	75,340	6,200,000				
9 *A*	2,450	100	11,800	6,580,000	2 hr.	− 690	45	9 ft. 8 in.
B	1,760	72	17,240	6,000,000				
10 *A*	1,390	100	7,200	9,230,000	3 hr. 10 min.	− 10	10	9 ft. 6 in.
B	1,380	99·3	15,700	8,432,000				
11 *A*	1,870	100	4,990	5,840,000	3 hr. 10 min.	+ 250	40	5 ft.
B	2,120	113	13,950	7,320,000				
12 *A*	1,480	100	8,600	7,784,000	3 hr. 5 min.	+ 980	35	5 ft. 2 in.
B	2,460	166	23,440	8,000,000				
13 *A*	2,310	100	9,760	—	3 hr. 10 min.	− 570	20	6 ft. 6 in.
B	1,740	75	17,100	—				
14 *A*	2,550	100	7,970	8,560,000	3 hr.	−1,100	20	8 ft.
B	1,450	57	19,630	8,640,000				
15 *A*	390	100	11,290	6,460,000	2 hr. 10 min.	+ 870	20	7 ft.
B	1,260	320	44,290	7,800,000				

Is there a marked change in the blood volume during these experiments? This is an important point, since the data obtained refer only to the lymphocyte count per cu. mm. of blood (see Table 2), whereas what we require to know is the change in the total number of lymphocytes in the blood as a whole. The slight loss of blood during the operation was immaterial, since the blood counts were not made until the operation was completed. After the operation, how-

ever, the possibility had to be considered of (1) a progressive post-operative shock, with loss of fluid from the capillaries, or (2) fluid leaving the capillaries to give rise to a peritoneal exudate. In either case the blood volume would diminish, and if this occurred to any considerable extent, there should be a rise in the red cell count. Using this latter count as a rough guide, the blood was found to show a slight tendency to become concentrated (Table 2), but the change was not constant. Peritoneal exudate was present in all cases, and

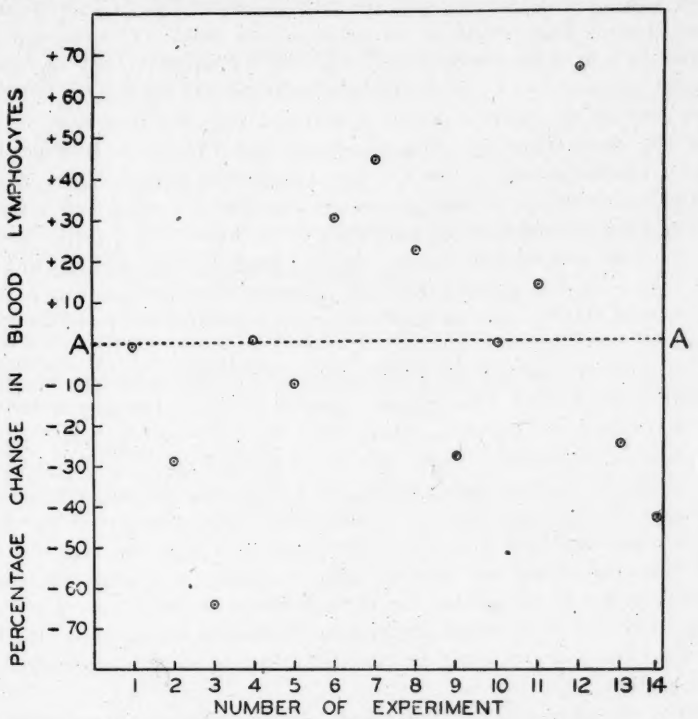


Fig. 1. Graph showing the percentage change in the blood lymphocytes between counts *A* and *B*. Count *A* is taken as 100 %, and is represented by the base-line *A*...*A*. Count *B* is represented by ⊙. There is no constant relationship between counts *A* and *B*. For further details see Table 2.

ranged—with one exception—from 4·0 to 45·0 c.c., with an average of 29·0 c.c., so that it could not be responsible for any marked change in the blood volume. The one exception was experiment no. 3, where the exudate was 95·0 c.c.; here, curiously enough, the red cell count actually fell, instead of showing the anticipated rise.

Table 2 shows that enterectomy is not followed by a constant change in the blood lymphocytes, but that they increase or decrease with about equal

frequency. From the argument presented at the commencement of this paper it might be supposed that an increase would most likely occur, whereas a decrease would be difficult to explain. Closer analysis shows that the reverse is the case, and that, of the possible changes in the blood lymphocytes following enterectomy, it is the increase which presents the real problem.

The vast majority of the lymphocytes reaching the blood are lymph-borne, i.e. they are conveyed to it by lymph flowing through the lymphoid tissues, whose newly formed lymphocytes are thus constantly being swept on into the blood stream. This lymph is derived from (1) limbs, (2) head and neck, (3) thorax, and (4) abdomen, finally reaching the blood through the thoracic or right lymph ducts. In the resting anaesthetized dog the number of lymphocytes entering the blood is greatly diminished, for there is no flow of lymph from the limbs (Starling, 1894), head and neck (Yoffey & Drinker, 1938), thorax (Drinker & Yoffey, 1941), or liver (Markowitz & Mann, 1931). The only lymph thus entering the blood is derived from the intestines, and it acquires its lymphocytes from the lymphoid tissue in the wall of the intestine and from the glandula mesenterica magna. In the anaesthetized animal, therefore, removal of the intestine and of the glandula mesenterica magna, as in the present experiments, cuts off the main supply of lymphocytes to the blood.

How will the cutting off of this supply affect the lymphocytes already present in the blood? This depends on what normally happens to them. If they leave the blood via the intestinal tract, then enterectomy and removal of the glandula mesenterica magna will at one and the same time both prevent new lymphocytes from entering the blood, and those already present from leaving it. In this case the blood lymphocytes would remain unchanged. But if they leave the blood in any way other than by way of the intestinal tract, then enterectomy will not interfere with the process of elimination, and the blood lymphocytes should fall, for there would be no fresh ones entering the blood to replace those which are leaving. Following enterectomy, therefore, the blood lymphocytes might be expected either to remain unchanged, or to diminish, but not to increase.

However, since in some experiments they do increase after enterectomy, the obvious question arises: Is there any way in which lymphocytes can enter the blood other than through the lymph stream? The spleen at once suggests itself as a possible source of additional lymphocytes, entering the blood directly, and not interfered with by enterectomy. Pending experiments in which enterectomy follows a preliminary splenectomy, this suggestion cannot altogether be ruled out. But while it is true that the spleen contains lymphoid tissue, and that earlier workers (e.g. Morris, 1914) believed, on the basis of comparative cell counts in the blood of the splenic artery and vein, that the spleen normally contributed a large number of lymphocytes to the blood, later work has discredited this view (Pearce *et al.* 1918). The experiments of Blalock *et al.* (1937) seem to show quite definitely that lymph-borne lymphocytes are

the chief source of these cells in the blood, for complete lymphatic obstruction resulted in the blood lymphocytes falling practically to zero.

SUMMARY

In fifteen dogs the major portion of the small and large intestine was removed. Within periods varying from 2 to 3 hr. afterwards, no constant change occurred in the blood lymphocytes. The mechanism of the control of the blood lymphocyte level is briefly discussed.

It is a pleasure to acknowledge my indebtedness to Major A. L. d'Abreu for his assistance in performing these experiments.

REFERENCES

BLALOCK, A. *et al.* (1937). *Arch. Surg.* **34**, 1049.

BUNTING, C. H. & HUSTON, J. (1921). *J. exp. Med.* **33**, 593.

DRINKER, C. K. & YOFFEY, J. M. (1941). *Lymphatics, Lymph, and Lymphoid Tissue.* Harvard University Press.

HOFF, P. (1934). *Ergebn. inn. Med. Kinderheilk.* **46**, 1.

LOEPER, M. & MARCHAL, G. (1922). *C.R. Soc. Biol., Paris*, **87**, 1081.

MARKOWITZ, C. & MANN, P. C. (1931). *Amer. J. Physiol.* **96**, 709.

MORRIS, D. H. (1914). *J. exp. Med.* **20**, 379.

NAEGELI, O. (1931). *Blutkrankheiten und Blutdiagnostik*, 5th ed.

PEARCE, R. M., KRUMBHAAR, E. B. & FRAZIER, C. H. (1918). *The Spleen and Anaemia.* Philadelphia and London: J. B. Lippincott Company.

STARLING, E. H. (1894). *J. Physiol.* **16**, 224.

YOFFEY, J. M. (1935). *J. Physiol.* **85**, 450.

—— (1936). *J. Anat., Lond.*, **70**, 507.

YOFFEY, J. M. & DRINKER, C. K. (1938). *J. exp. Med.* **68**, 629.

Anatomical Society of Great Britain and Ireland

PROCEEDINGS

July 1941

RECORDED BY

A. J. E. CAVE

PROCEEDINGS. ANATOMICAL SOCIETY

JULY 1941

The Annual General Meeting of the Society for the Session 1940–1 was held in the Anatomy School, New Museums, Cambridge, at 2 p.m. on Friday, 4 July and at 9.30 a.m. on Saturday, 5 July 1941, with the President (Professor J. P. Hill) in the Chair.

Dr W. F. Harper read a paper on 'The blood supply of the human ureter'.

Professor Gray referred to barium emulsion injections made by the late Professor Woollard and himself in human foetuses, which confirmed the present picture of the blood supply of the pelvis and upper two inches of the ureter; the injections had failed to demonstrate any ureteric branches from the spermatic artery; ureteric lymphatics were extremely difficult of demonstration. Professor Le Gros Clark and the President questioned the absolute validity of the intra-epithelial ureteric vascular plexus described. Dr Bacsich referred to Abraham's work on the intra-epithelial capillaries of the rabbit bladder and to the existence of a similar capillary system in the guinea-pig bladder. In reply, Dr Harper explained further the construction of his picture of ureteric capillary arrangements: intra-epithelial capillaries were undoubtedly present in the cat and rabbit as in the human subject.

Professor H. A. Harris read a paper on 'The veins of the suprarenal, kidney and ureter'.

The venous anastomoses among the renal, capsular, spermatic, inferior phrenic and azygos systems known and described from the time of Eustachius were enumerated in detail. Illustrations were shown from the older anatomical writers, together with modern pictures based on the employment of opaque vascular injections and radiography; the embryological and clinical significance of the various anastomoses was described. A thin peripheral zone of renal cortex was vascularized from the capsular artery.

Professor Kirk commented on the primitive vascularization of the common mesodermal sheath of the developing intermediate cell mass organs in relation to the variety of permanent vascular connexions later established. Professor Cave referred to a fairly constant anastomosis between the inferior mesenteric and ureteric veins.

Dr W. L. H. Duckworth read a 'Note on Greek and Cretan skulls'.

Human crania from the Monument at Chaeronaea were among the specimens in the museums of Athens reported on in 1903. Renewed study of the notes then made has led to the surmise that one of these Chaeronaea skulls may have its counterpart in Swedish stone age (neolithic) examples.

Dr W. L. H. Duckworth read a paper on 'A foetal rhinoceros'.

The specimen described, a foetal African rhinoceros, now 18·4 mm. in length, measured 19·3 mm. when first received, after immersion in 70% alcohol for a considerable period. Externally, the specimen was compared with a pig foetus, fixed and preserved in formol, but somewhat larger (26 mm.): the difference in size probably reflects the contrast in the preservatives used.

Dr W. L. H. Duckworth read a paper on 'The Andamanese aboriginal brain in the Cambridge collection'.

The study of this Andamanese brain was rendered difficult by the fact that, though well preserved, it was distorted through careless packing, so that the frontal region

appears unduly large and the occipital poles unduly attenuated. To correct this distortion in a drawing of the specimen the following artifice was adopted. The outline of an endocranial cast of an Andamanese skull of corresponding size was drawn upon a sheet of extensible rubber: this sheet was then stretched till the endocranial contour tallied with that of the distorted brain and only then, on the sheet so stretched, were the principal sulci drawn. The rubber sheet was then released, and being relaxed, the sulci so drawn upon it appeared as if drawn upon the outline of a normally shaped hemisphere: their comparison with those of other brains was thus facilitated.

Professor Le Gros Clark commented appreciatively upon this excellent and novel method of reconstructing the sulcal pattern of a deformed brain. Dr Duckworth added that the Andamanese specimen was a well-constituted brain, presenting a pronounced asymmetry of its hemispheres.

Dr P. Bacsich read a paper on 'Nerve cells in the myenteric plexus of the tench', of which the following is an abstract:

A unique feature of the alimentary canal of the tench (*Tinca vulgaris*) is its investment, throughout its entire length, by a double coat (an outer longitudinal and an inner circular) of striped muscle, innervated by the vagus, which forms a rich nerve plexus between the two layers. Within the striped muscle coat is the tunica muscularis propria, composed of an outer longitudinal and an inner circular layer of unstriped muscle. The myenteric plexus is situated in the longitudinal unstriped muscle layer, as well as upon the internal and external surfaces thereof. The presence of nerve cells in the myenteric plexus has not hitherto been convincingly demonstrated: the usual silver impregnation methods, while demonstrating an extremely rich network of nerve fibres, yield consistently negative results. The matter is of some moment for, should nerve cells be absent in any particular species, the myogenic nature of peristaltic movements in the isolated intestine of that species must be assumed.

In the tench, by a combination of the Bielchowsky-Gros silver technique and toluidin-blue staining, the presence of nerve cells has been established, and their cytology studied. The cells are rather small (10–15 μ) multipolar cells: the cyto-architecture of the myenteric plexus reveals no definite ganglia, but an abundance of scattered nerve cells.

Neurological and histological considerations suggest that a ganglionated plexus has been erroneously regarded as the myenteric plexus, whereas it is either the condensation of myenteric and submucous plexuses, or, more likely, nothing more than the submucous plexus of a rather pronounced muscularis mucosae.

The President remarked upon the peculiar difficulties encountered in the staining of nerve cells in the tench, a relatively easy matter in many other fresh-water species.

Mr E. W. Walls read a paper on 'The cardiac conducting system of the golden hamster (*Cricetus auratus*)'.

Messrs E. C. Amoroso, A. E. Barclay, K. J. Franklin and M. M. L. Prichard read a paper on 'The nomenclature of the great veins and heart of the eutherian foetus', of which the following is an abstract:

(1) The umbilical venous inflow dominates the so-called 'portal' sinus; it is suggested that this name be replaced by the name 'hepatic sinus'.

(2) Great difficulty is encountered in describing accurately the circulation through the foetal heart and great veins if the present purely morphological terminology of the parts concerned be adopted. In the light of personal anatomical and physiological findings the following nomenclatorial scheme is advanced: The posterior (or inferior) caval channel enters the heart and therein divides on a prominent crest, the crista dividens, into right and left terminal divisions. This last is bounded by a fixed portion (i.e. part of the inter-atrial septum and perhaps other structures) and a free or apposable portion (i.e. the so-called valve of the foramen ovale, which in many genera

is most unlike a valve). After birth the apposable portion is apposed and the left terminal division ceases to exist. The right terminal division is separated dorsally from the anterior (or superior) caval channel by the tuberculum intervenosum of human, or the crista intervenosa of veterinary, anatomy, for which structure the term crista interveniens is suggested.

The PRESIDENT and Professors BOYD, CAVE and HARRIS briefly discussed this communication. Sir JOSEPH BARCROFT emphasized the valvular nature of the foramen ovale and the changes undergone by it during the cardiac cycle: he considered the authors had made a great advance by rendering intelligible the crossing of the cardiac blood-streams.

Dr W. F. HARPER read a paper on 'The vascular content of human heart valves', which presented a summary of present knowledge regarding the vascularity of these structures in both health and disease. Cell types in the subendothelial zone of normal human atrio-ventricular valves of different age periods were described and comparisons established with corresponding types in normal rabbit heart valves.

The PRESIDENT, Professor LE GROS CLARK and Professor GRAY commented on this paper.

Dr W. F. HARPER read a paper on the 'Vital staining capacity of normal and of damaged heart valves in the rabbit'.

Professor J. D. BOYD read a paper on 'Vascular pressor-receptors in *Pelias berus*'.

The PRESIDENT congratulated the author upon the quality and import of the matter of this communication. Mr W. E. ADAMS discussed particular points; the ophidian so-called carotid body was more properly an epithelial body.

Drs P. BACSICH and G. M. WYBURN read a paper on 'Observations on artificially induced heat in immature guinea-pigs', of which the following is an abstract:

Twelve immature female guinea-pigs were brought on heat by injections of oestrin and progesterone. The animals were killed and their uteri sectioned, stained (some few with haemalum and eosin, the remainder with the benzidine stain) and examined histologically. The uteri of three untreated immature animals were also sectioned and similarly stained, and used as controls. The uteri of the treated animals were greatly enlarged, the endometrium exhibiting proliferation, branched glands, etc., as in a normal uterus 'on heat'. In every case, however, there was observed an absence of the characteristic antimesometric hyperaemia to be found in the uterus of the adult intact animal during oestrus or in the spayed female during induced heat. In contradiction to the suggestion that the hormonal response of the immature uterus is complete and characteristic, it is suggested that the present negative findings provide concrete evidence in favour of the conclusion that failure to induce implantation in the immature animal is due to an inadequate preparation of the endometrial bed.

Professor HAMILTON enquired whether ovulation had been brought about in the animals used. Dr BACSICH replied in the negative: with oestrin and progesterone neither true ovulation nor follicular growth had been observed, although ovulation without subsequent successful implantation had been recorded by other workers: gonadotropic hormones would next be employed in the continuation of the present studies.

Professor W. E. LE GROS CLARK read a paper on 'The laminar organization and cell content of the lateral geniculate body in the monkey'.

In the subsequent discussion the author answered affirmatively Professor BOYD's enquiry as to whether the anterior end of the lateral geniculate receives fibres from each retina. Dr WEDDELL asked whether the triple-layered nature of the lateral geniculate was in any way correlated with the physiology of colour vision. In reply

it was stated that a three-fibre conducting unit from the retina had been established, such as was implied by the Young-Helmholtz trichromatic theory of colour vision, and details were given regarding the conditions of the lateral geniculate body and the associated degrees of chromatic vision observed in various South American monkeys.

Dr G. WEDDELL read a paper on 'The multiple innervation of cutaneous sensory spots'.

Drs G. WEDDELL and P. GLEES read a paper on 'The early stages in the degeneration of cutaneous nerve fibres'.

The PRESIDENT commented upon the curious and singular phenomenon described of simultaneous regeneration and degeneration in normal nerve trunks. Mr W. E. ADAMS was answered that trauma certainly played a causative part in this phenomenon. Professor GRAY emphasized the value of such anatomical studies as those presented as a sound basis for physiological theory. Dr WEDDELL replied to the discussion.

Sir JOSEPH BARCROFT (introduced by Dr K. J. FRANKLIN) exhibited a cinematograph film depicting the incidence and crescence of body movements in the foetal sheep.

Mr G. W. HARRIS read a paper on 'The application of remote control stimulation to the hypothalamus', of which the following is an abstract:

Loucks (*J. comp. Neurol.* 1934, **18**, 305) and others have described techniques for stimulation of the central nervous system in the unanaesthetized animal by the implantation beneath the skin of a solenoid wherefrom an electrode led to the particular tissues to be stimulated. In such experiments the motor cortex was the usual area chosen for stimulation, which was effected by placing the animal's head and the inserted coil in an electromagnetic field. It became desirable to stimulate the hypothalamus in an animal which had received no anaesthetic during the days immediately before the experiment, and also to be able to repeat the stimulation at intervals throughout a prolonged period. A technique was therefore devised to subserve such purpose. A hollow German silver screw is inserted into the animal's skull at the bregma: a coil of copper wire, with an electrode of silver wire insulated by glass capillary tubing to within 1 mm. of its tip, is racked down by means of a stereotaxic instrument (Harris, *Proc. Roy. Soc.* B, 1937, **122**, 374), so that it fits tightly in and around the previously inserted screw. The stimulating electrode passes through the screw, the meninges and the corpus callosum, its tip being finally placed in the region of the tuber cinereum. The indifferent electrode is an oval German-silver plate attached at the periphery of the coil and situated on the dorsum of the animal's nose. The whole coil is covered with paraffin wax before the skin is sutured: its position is controlled by periodic X-ray examination.

This technique has been employed to date upon eight animals (rabbits). Of these, two died at operation (one from intra-ventricular haemorrhage, one from anaesthetic overdose), one was killed two and a half months after operation because of sepsis in the wound, and five remain alive and healthy at seven, three, three, two, and one month periods respectively after operation.

The PRESIDENT asked how particular hypothalamic nuclei were attacked by the technique described. Professor HARRIS referred to the simplicity and cheapness of the technique, its advantages over the Clarke-Horsley apparatus, its discarding of anaesthesia and its suitability for the study of particular problems. In reply, the author indicated that the technique advanced, which involved the use of an alternating current, was designed, not for the destruction of particular hypothalamic areas, but for stimulation of a functional area: for destructive lesions the Clarke-Horsley apparatus and the direct current were preferable. The great advantage of the present method was its elimination of anaesthetics, with their attendant dangers and difficulties, and the feasibility of repeated stimulation of the same cerebral area.

Mr D. V. Davies read a paper on a 'Case of ectopia vesicae with amputation of one hind limb'.

In the discussion following, the President doubted whether the long tubular structure interpreted as neurenteric canal was correctly so identified: he thought it more probably to be partly neurenteric canal and partly post-anal gut. Dr Wyburn stressed the importance of experimental embryology as the key to the reasons for deficiencies of tissues and organs: in many such deficiencies (harelip, cleft palate, etc.) the mesoderm was the faulty tissue. Perhaps the present case exemplified a primary mesodermal faultiness. Dr Bacsich enquired whether the single umbilical artery present reached the placenta, and was informed that no placenta accompanied the specimen. Mr Adams asked about the position of the ureters, which were normal, though the uterus was double and a vagina wanting: he considered the case one of extroversion of the cloaca rather than of the uro-genital sinus. Professor Harris remarked that in 75 % of cases of interference with the hind-limb but a single umbilical artery to the placenta was to be observed.

Mr G. J. Romanes read a paper on 'The spinal cord of a case of ectopia vesicae with amputation of one hind limb'.

Mr Walls commented upon the association of muscle groups and spinal cord cell groups as manifest in the specimen described: the posterior columns showed marked asymmetry and the absence of the one hind limb was reflected in the architecture of the cord. Replying, the author considered the deficiency of the posterior columns to be due rather to their intrinsic nature as neurones of the first order.

Mr S. Wilson Charles read a paper on 'Madder experiments and the growth of bone', of which the following is an abstract:

Madder experiments are almost universally accepted as indicating the method whereby bones grow, but it is by no means certain that the usual interpretations of such experiments are correct. There appears no justification whatever for the assumption that the supposed method of growth of the cranial bones of the pig at 4–10 months of age gives any indication of the corresponding growth in the foetal or the young pig, still less of growth in the human foetus. Recent examination of developing cranial pig-bones shows that these do not grow in the manner imputed to them by the customary interpretations of madder feeding experiments, and that the cranial membrane bones do not undergo increase or expansion by a process of re-formation. In support of these statements diagrams were demonstrated of material from pig specimens varying in age from the foetal (commencement of ossification) to the 3-month-old animal.

Professor Harris, employing slides and radiographs in support of his remarks, referred to details of cranial growth in the foetal pig and to the experimental work of Hunter, Keith and Brash: Hunter's distinction between 'red' and 'pink' bone was often overlooked in the interpretation of madder feeding results, themselves not infallible, and frequently misinterpreted. Dr Haines did not regard the criteria of madder experiments as altogether valid because of the impossibility of establishing satisfactory comparisons between animals of different species or different ages. The pig was, in most respects, a highly specialized and peculiar type, and it was illogical to assess human ossification patterns from pig material and vice versa. The parietal bone certainly grew by accretion upon its outer and inner aspects as well as by increase at its sutural borders. Professor Le Gros Clark discussed cranial ossification in the human foetus and the variations of ossificatory pattern encountered: he considered that the madder method in the post-natal pig probably did afford some indication of the mode of bone growth in the foetal animal. He asked what were the criteria for the absorption and the deposition of new bone. In reply the author said that, in the pig's parietal, growth occurred over a large area and the bone did not enlarge by simple re-formation.

Professor W. J. Hamilton and Dr R. J. Gladstone read a paper on 'A presomite human embryo (Shaw), with primitive streak, chorda canal, etc.'

The President discussed various features of this embryo. He doubted the validity of the evidence submitted regarding the direct entodermal origin of blood cells: in some mammals an entodermal origin of such seems probable, but in general, the primitive blood cells arise from mesoderm. Dr Wyburn discussed the present embryo in relation to others previously described, particularly the McIntyre embryo, in which Bryce had observed certain lateral cavities of unknown significance at the front end of the prochordal plate: no such cavities appeared in the Shaw specimen. Blood vessels might be equally entodermal or mesodermal in origin, and Streeter had emphasized the extreme difficulty of any early distinction between entoderm and mesoderm. In reply the authors dealt with points raised in the discussion and referred to Wang's work on the ferret which supported the entodermal origin of the blood cells.

Professor W. J. Hamilton read a paper on 'Some observations on the early development of the cow'. This communication dealt with living unsegmented and segmented cow ova recovered from the uterine tube at various times after ovulation and after insemination. The successive stages of development of the cow egg were illustrated by photographs of these living specimens.

Mr D. M. Samuel read a paper on 'The early development of the golden hamster', a preliminary communication dealing with the morphology and development of the unsegmented and segmented eggs of this species, after recovery from the uterine horn and tube. The golden hamster ovum is of small size, reaches the uterine horn at a four-cell stage, and soon becomes enfolded in a pocket of the cornual mucous membrane. The paper was illustrated by photographs of the developing ovum from the period of its fertilization up to the time of blastocyst formation.

In the subsequent discussion the President commented on the marsupial-like shortness of gestation (16 days) in this species and upon the curious inequality of size of the cells of normal hamster segmenting eggs, a condition recalling the similar inequality observed by Hamilton in the ova of the goat and ferret.

Dr Amoroso congratulated the author upon his excellent series of ova, which in many ways resembled the ova of the mouse, and suggested means facilitating their future procuration.

Dr A. M. Carleton read a paper on 'A comparative study of the inferior tibio-fibular joint'.

Commenting on this paper Dr Haines disagreed with the statement that fishes lacked diarthrodial joints, for in that class such joints were always present where the articulating bones were of large size, e.g. mandible, branchial arches: diarthrodial joints were part of the primitive tetrapod inheritance.

Dr R. W. Haines read a paper on 'The knee-joint of tetrapods'.

In the subsequent discussion Professor Harris detailed the ossifications present in the knee-joint menisci of various common animals. Professor Kirk said the paper explained the apparent anomaly of epiphysial ossification in the human fibula. Dr Carleton thought that if rotation at the knee joint was, primitively, of greater amplitude than at the ankle joint, then difficulties arose in explaining the distribution and attachments of muscles in the tetrapod hind-limb. Dr Haines replied, dealing particularly with muscle attachments to tibia and fibula and their effect upon movements at the ankle joint in both reptiles and mammals.

Messrs E. C. Amoroso, W. F. B. Griffiths and W. J. Hamilton read a paper on 'The development of the goat during the first seven days', of which the following is an abstract:

The detailed course and the rate of development of the goat egg during the first

week were shown. The observations were based both on the study of the living egg and on that of serial sections of eggs. Particular attention was called to the diverging rates of differentiation of the egg and to the mutual inductive influence of the cell groups thus created. By the 6th day the egg appears to be definitely organized as to its axis; an embryonic pole of many cells is distinguishable, also an abembryonic pole of fewer cells. At least four types of cells are recognizable: (1) primitive formative cells, (2) primary entoderm cells, (3) covering trophoblast cells, and (4) parietal trophoblast cells.

The PRESIDENT remarked upon the great difficulties of interpretation in very early embryonic stages; he doubted whether the covering layer of trophoblast did derive from proliferation of central cells as suggested, and did not regard the sectional evidence submitted as wholly conclusive of the authors' contention in this matter. He regarded the trophoblast as a distinctive entity. He suggested the better fixation of future material by the immediate transference of the eggs to Fleming's solution. Professor HAMILTON replied to the points raised, remarking that the specimens already studied had been fixed in Fleming's solution and dealt with by a technique elaborated by Professor HILL himself.

DEMONSTRATIONS

At this Meeting of the Society the following Demonstrations were given:

(1) A. E. BARCLAY, K. J. FRANKLIN *et al.*: X-ray cinematographic studies of circulation in foetal sheep after Caesarian delivery.

(2) A. E. BARCLAY, K. J. FRANKLIN *et al.*: Comparative anatomico-physiological studies of cardiovascular system of eutherian foetus.

(3) W. J. HAMILTON: Specimens of early implantation in the golden hamster.

(4) D. M. SAMUEL: Living ovum of the golden hamster.

(5) W. J. HAMILTON and R. J. GLADSTONE: Presomite human embryo (Shaw).

(6) P. N. B. ODGERS: A presomite human embryo.

(7) P. BACSICH: Nerve cells in the myenteric plexus of the tench (*Tinca vulgaris*).

(8) G. WEDDELL: Methylene blue preparations illustrating cutaneous nerve plexuses.

(9) C. F. V. SMOUT: Model of cranial nerve nuclei.

(10) W. E. LE GROS CLARK: Reconstruction model of lateral geniculate body of monkey. Cast of skull of *Plesianthropus*.

(11) P. GLEES: Optic fibre terminations in lateral geniculate body of cat and rabbit.

(12) W. F. HARPER: Specimens showing blood supply of human ureter. Specimens showing structure of human and rabbit cardiac valves.

(13) G. J. ROMANES: Comparative anatomy of motor cell groupings in cervical spinal cord. Spinal accessory nuclei in certain mammals.

(14) A. T. PHILLIPSON: Radiographic studies of sheep stomach.

(15) H. A. HARRIS and G. D. WEST: Apparatus to show effect of arterial pulsation on lymph drainage.

(16) H. A. HARRIS: Radiographic studies of embryos. Specimens and models illustrating vertebral growth.

(17) D. V. DAVIES: Ectopia vesicae with persistent neurenteric canal.

(18) J. HAMMOND: Corpora lutea of the cow.

(19) E. C. AMOROSO *et al.*: Tubal and uterine ova of the cow. Cleavage of the goat egg.